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A NUTRITIONAL STUDY OF INSECTS, WITH SPECIAL REFERENCE TO MICROÖRGANISMS AND THEIR SUBSTRATA[1]

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[1] Contribution from the Entomological Laboratory of the Bussey Institute, Harvard University.

INTRODUCTION

Throughout the whole organic world the essential food element most difficult to acquire is nitrogen, as all nitrogen must ultimately come from the atmosphere and the power of combining with this gas is limited to a few microörganisms. Upon the nitrifying bacteria, then, all higher plants and animals are dependent for their nitrogen which is handed from one organism to another, linking all together into one great interdependency which has

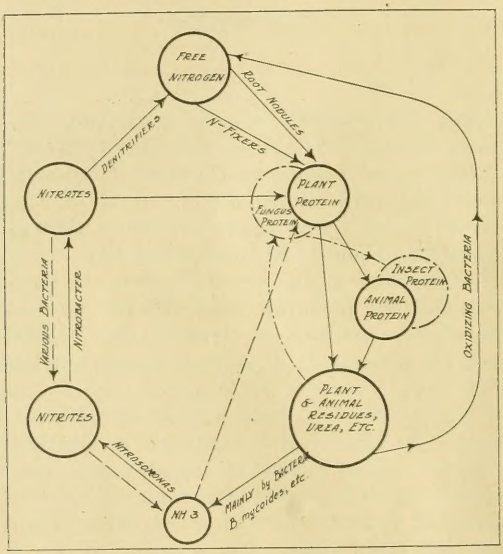


Fig. 1 The nitrogen cycle (from Bayliss). The accessory lines and circles in — - — - — are my additions based on evidence in this paper.

been called the nitrogen cycle. I insert a diagram from Bayliss which clearly illustrates this cycle. The accessory circles and the lines that connect them are additions based on my experiments.

The search of the insect for nitrogen is very complicated and has been, at times, obscure. Indeed, little definite information is at hand concerning the food requirements in general of these organisms, as the material consumed is often in large part merely the substratum for a small amount of assimilable food. This has led to many misunderstandings as to the synthetic power of

insects. Since they are largely phytophagous, insects are amply supplied with carbohydrates, but have difficulty in obtaining sufficient protein. The abundance of the former permits great activity, while the dearth of the latter limits the growth of the insect. This has led to a lengthening of the life-cycle in those species which must ingest large quantities of substrate in order to get enough nourishment to complete their growth. However, many insects that feed in decaying of fermenting vegetable matter of low protein content have an unusually short period of growth. The experiments and considerations which follow throw light on the protein supply of such insects and account for their rapid growth.

These investigations were made at the Bussey Institution for Research in Applied Biology, Harvard University, under Prof. W. M. Wheeler. Valuable advice and assistance were received from Profs. C. T. Brues, W. J. V. Osterhout, I. W. Bailey, and Dr. R. W. Glaser. I am especially indebted to Doctor Wheeler for helpful suggestions and encouragement.

EXPERIMENTS

1. Food of an insect (Drosophila) living in fermenting fruit

A. Method and initial observations. *a.* Solid media for Drosophila. While rearing Drosophila it was found necessary to determine the exact date of oviposition. As this is impossible in the ordinary culture tube of fermenting banana, a solid transparent medium was devised by myself and Dr. R. W. Glaser (1917 a).

This medium is made as follows: Mash six ripe bananas in 500 cc. of water, allow to infuse on ice overnight, strain through cheese-cloth, and add $1\frac{1}{2}$ grams powdered agar-agar to each 100 cc. of the filtrate. Heat in double boiler till agar is dissolved, filter hot through absorbent cotton into test-tubes. Plug tubes, sterilize in autoclave, and allow to cool in inclined position so as to form solid slants of the medium.

This medium is quite transparent, affords 15 to 20 sq. cm. area for oviposition and 6 to 10 cc. of substratum for the larvae. The

eggs, which are readily deposited by the female, are prominent objects on the agar.

Bacterial and fungous growths occur over the surface, but I noticed that unless these become too luxuriant before the larvae hatch, they are destroyed by the insect.

The agar method has the advantage of permitting observation of the date of egg deposition and hatch and the details of larval habits. It also furnishes a method of making nutritional studies of various synthetic media.

b. Preliminary observations on the food of Drosophila. In May, 1916, while rearing Drosophila melanogaster on banana agar, I noticed that molds and bacteria often completely covered the surface of the medium and killed the larvae. This was confined to cultures which had only ten or twenty instead of the usual fifty or a hundred larvae. The larvae congregated at the points where fungus was most abundant and caused the plants to disappear, apparently by feeding upon them.[2] An examination of the flora showed that Saccharomycetes were invariably present and often occured in pure cultures.[3]

This observation suggested an internal symbiosis between Drosophila and yeast. I found, nevertheless, that by washing the surface of the pupae with alcohol, the insect could be freed from all microörganisms. The larvae of such sterile insects were not able to mature on banana agar nor could they mature on a synthetic medium of salts and sugars with ammonium tartrate as the source of nitrogen, as had been maintained by Loeb ('15[4]), but were able to develop on either medium in the presence of yeast cells.

c. Habits of adults and larvae. The Drosophila were introduced as pupae, usually three being placed on the side of the test-tube. The adults emerge after five to eight days, the time depending on the temperature, and readily feed on the banana medium,

[2] This interpretation was first suggested to me by Mrs. J. Jackson.

[3] In 373 transfers of pupae, all descendants of adult Drosophila, taken from a stock bottle of fermenting banana, all tubes were infected with yeast cells carried on the bodies of the insects.

[4] Loeb has since corrected this view ('16). Loeb and Northrop ('16 b).

on which they leave little depressed spots where they have regurgitated and sucked up the dissolved substance. If the medium has not dried enough to have taken on a hard, leathery crust, the females oviposit after twenty-four hours and continue to do so for some days. The eggs are thrust into the agar so that the upper end with its two projecting floating structures is just level with the agar; in this position they are prominent objects under the binocular. After a period of one or more days, the minute larvae leave the eggs and move about over the surface of the medium. They are at this time usually 1.2 mm. in length. By the second day they have increased in size to 1.8 to 2 mm. in length, and begin to work in a vertical position, with the anterior end down, the full length of the body in the jelly, and the posterior end with its two projecting spiracles either in contact with the air or with a bubble of air which has been enclosed in a thin film of the medium and remains attached to the larva, thus enabling the latter to work the food material to a greater depth than its body length would permit. The head end of the larvae is merely a small pointed segment which served as a collar through which the pseudo-maxillary apparatus works. In shape the latter may be roughly compared to a plow with the shares prolonged posteriorly into two handles. Attached at the anterior end of this four-pointed median structure, is a pair of deflected falcate processes, sharp at the point and on the concave side, that work up and down constantly with a simultaneous backward and forward movement of the whole apparatus. The movements of these oral organs were observed in a drop of agar on a depression slide, and it was found that their constant movement continued without any appreciable rest periods. Occasionally the movement would stop without apparent reason for about two minutes, but there was no regularity in these periods of cessation. The larva might work for fifteen minutes without stopping or might stop several times at intervals of two or three minutes. Apparently the recovery from fatigue takes place in the interval between the movements. Progression of the larva seems to be due to a series of protrusions of the anterior end with an accompanying circular contraction, the animal being held in place by the circles of spines on each seg-

ment, while the posterior end is drawn up. In more fibrous material, the mouthparts probably aid the larva in moving about. When fully grown, it leaves the medium to pupate on the side of the test-tube or the surface of the medium itself.

d. Ecology of cultures. Drosophila is very extensively used by geneticists in breeding experiments. The insect is reared in small glass bottles or milk jars, plugged with cotton and containing fermenting banana covered with absorbent paper. Quite often these 'cultures go bad,' i.e., smell strongly of acetic acid or become putrid or covered with mold, so that the insects are destroyed and the breeding experiment terminated.

The method commonly employed in making the culture media is to boil skinned bananas, to cool the mass and to add two cakes (24 grams) of Fleishmann's bread yeast (bottom yeast) per dozen bananas. This is allowed to ferment and is used as a stock supply from which to prepare clean culture bottles. In this manner the medium is kept fairly sweet, probably due to the great development of the yeast, with an accompanying production of alcohol which retards[5] the development of molds and bacteria.

If pupae are taken from a bottle that has gone 'bad' and placed on banana agar, a number of different bacteria or molds may develop around then, prominent among which are a mucor, Rhizopus nigricans Ehrenberg, the bread mold, Aspergillus, the green herbarium mold, Penicillium glaucum, the blue mold, and the acetic acid bacillus. If pupae are taken from a good culture tube with yeast alone or yeast and the acetic acid

[5] In this connection Lafar ('10, II, 2, pp. 238–240) writes: "From the standpoint of the oecological theory of fermentation, the alcohol produced by yeast should be regarded as a weapon capable of hindering the appearance of other fungoid competitors in saccharine nutrient media. However, when accumulated in the medium during the progress of fermentation, it also restricts the further development and action of its producer. In this case, as with yeast poisons in general, the first result is the cessation of cell reproduction, a larger quantity of alcohol being necessary to arrest fermentation and a still further quantity to kill the cells." Reproduction of yeast cells ceases at a 6 per cent and fermentation at a 5 to 24 per cent concentration of alcohol. It should be also remembered that most bacterial or fungus cultures have a tendency to become pure, probably owing to the production of some definite antagonistic substance, or to better adaptation to the medium by the successful form (Hiss and Zinsser, '10).

bacillus, the Drosophila larvae grow rapidly, the fungous growth soon diminishes and is visible at only a few points on the surface. If the flora contained molds, the whole surface of the medium is soon covered and the Drosophila eggs are killed, or more often hatched and the young larvae die. If the mold does not completely cover the surface, many larvae survive, and upon increasing in size, are able to destroy the mass of mold hyphae and form a fairly clean surface. The larvae are able to do this only when they are in large numbers and have reached a size of 3.5 to 4.5 mm. before being covered by the molds. It would seem, therefore, that the destructive action of the molds is mechanical rather than toxic. It was also observed that molds seldom gain a foothold on media in which large numbers of larvae are feeding. This observation explains why 'strong' cultures of Drosophila (as usually reared on fermenting banana) remain 'sweet' and seldom go bad. Banana-agar culture tubes in which the Drosophila pupae have been placed on the glass, rather than on the medium itself, often remain sterile till the adults emerge. The latter spread the spores over the surface of the agar at the same time that they deposit their eggs. Thus the molds and bacteria have little time to grow before the larvae are at work.

The development of molds and bacteria is not apparent in the presence of large numbers of larvae and a strong culture of yeast in the proper nutrient medium.

e. Media for genetical work. In selecting the best medium in which to rear Drosophila the most important considerations are abundant food for the yeast cells and a moist jellylike consistency of substratum to which the larvae are adapted. Transparency and solidity of media will add to the convenience of the investigator.

I have obtained the best results by using Saccharomyces ellipsoideus, in the stock bottle of banana, as the fragrant odors of fermentation produced by this yeast stimulate oviposition by the fly. The two following media have proved most satisfactory: 1. Fermented banana agar. Ferment one dozen mashed bananas for 48 hours, strain through cheese-cloth, add agar, sterilize and slant. 2. Pasteur's culture fluid agar.

10 grams ashes of yeast
10 grams ammonium tartrate
100 grams rock candy
1000 grams water

Add agar, sterilize in Arnold sterilizer, slant.
Into sterile tubes of these media the introduced adults or pupae carry living yeast cells which are distributed through the medium by the activity of the larvae.

f. Are living yeasts present in the egg or pupa? In the following experiments undertaken to show that microöganisms are not transmitted through the egg of Drosophila, the first precaution was to free the insect from external microörganisms. Usually eggs are used for this purpose, but the small size of Drosophila eggs makes this a difficult procedure. As it is well known that the lining of the digestive tract of larvae is thrown off upon pupation, pupae were selected for sterilization.

The pupae from a culture strong in yeasts were submerged in 85 per cent alcohol for ten minutes and then introduced aseptically into sterile slant culture tubes of agar-agar and fermented banana filtrate. If no yeast developed around the pupae which were placed on the food, the tube remained sterile after the emergence of adults, oviposition, and hatching of larvae. The sterility of the tube was later tested by introducing a few loopfuls of the medium into a sterile tube of similar food. It had previously been determined that yeast developed readily on fermented banana agar.

2. Larvae which had been feeding on media containing living yeast cells were submerged and washed in 85 per cent alcohol and then introduced into sterile culture tubes. In all cases yeast developed on the new media. Cultures from the digestive tracts of the larvae gave similar results. Apparently, many cells escape digestion in the stomach, as is the case with seeds or insect eggs in birds.

3. Eggs were sterilized by soaking in 85 per cent alcohol for ten minutes. The larvae which hatched were always sterile.

From the foregoing experiments we may conclude that living microörganisms are not present in the eggs or pupae of Drosophila.

However, a loose symbiosis exists between yeast and the insect. As mentioned above, surface fungous growths disappear in the presence of larvae which often seemed to be more numerous at this point. From these observations I inferred that the larvae fed upon the microörganisms present.

g. Sterilization of pupae. The sterilization was accomplished by the use of ethyl alcohol. As a precaution the operator's hands were washed in alcohol, and a lighted burner, clean forceps and platinum loop as well as sterile culture tubes were ready on a

TABLE 1

CULTURE NO.	NO. PUPAE	ALCOHOL TREATMENT	NO. ADULTS EMERGED	LARVAL PERIOD	CONTAMINATION
A 8	2	50% 10 seconds	2	None	Yeast cells
A 9	2	50% 5 seconds	2	None	Yeast cells
A 10	3	50% 20 seconds	3	12 days p[1]	Yeast cells
A 11	3	50% 2 seconds	3	11 days p	Yeast cells
A 12	3	50% 2 seconds	3	14 days p	Medium brown, yeast coccus, rod
A 13	2	50% 2 seconds	1	None	Yeast cells (?)
A 17	3	85% 2 minutes	3	26p	Yeast cells
A 18	8	85% 5 minutes	6	28d[1]	
A 19	5	85% 6 minutes	5	None	
A 20	6	85% 7 minutes	6	26p	Yeast cells
A 24	5	85% 10 minutes	4	25d	
A 25	7	85% 10 minutes	6	44d	

[1] d indicates larval death
p indicates pupation

table also washed with alcohol. Pupae were taken from a tube having a strong growth of yeast, but uncontaminated by molds[6] and placed in a sterile watch-glass. Alcohol was then poured in till the pupae were submerged. All floating pupae and all larvae were removed. The results of this treatment for different periods of time are shown in table 1.

The pupae are able to withstand a treatment of 25 minutes in 85 per cent alcohol if applied when they are about two days old.

[6] The frequency with which pure yeast growths occur in Drosophila cultures has already been mentioned on page 4.

Treatments of five minutes seldom kill the pupae, and in 90 per cent of the cases render them sterile. The sterilizing effect was not entirely understood till pupae were used which came from a Drosophila stock bottle of fermenting banana contaminated by molds and bacteria. These pupae when washed with 85 per cent alcohol saturated with $HgCl_2$ were sterilized in less than 50 per cent of the cases as shown in table 2.

This indicates that the sterilization involves two stages, 1) destruction of molds and bacteria by feeding of the larvae and a good strong yeast growth and, 2) killing of yeast by alcohol.

The toxicity of alcohol for yeast cells is shown to be high by the following experiments:

Three grams of yeast were separated in 25 cc. of sterile water and two drops of this fluid were added to each of ten watch-glasses filled with 85 per cent alcohol and to ten sterile banana-agar tubes.

TABLE 2

NO. CULTURES	NO. PUPAE	TREATMENT	NO. ADULTS EMERGED	NO. TUBES CONTAMINATED	NO. PUPAE CONTAMINATED
15	150	85 per cent alcohol sat. with $HgCl_2$	15	8	83±

After 1, 5, 10, 15, and 20 minutes, respectively, two sterile banana agar tubes were inoculated with two drops of yeast from the watch-glasses of alcohol. The tubes were kept under observation for 21 days. The results in table 3 show that a five-minute exposure to 85 per cent alcohol is fatal to yeast cells.[7]

h. Test of sterility. The sterility of a culture tube could usually be judged by the fact that no growth occurred, 1) around the pupae which were placed on the medium, 2) at spots where adults regurgitated on the medium, 3) at adult fecal spots, 4) upon oviposition, 5) upon emergence of larvae, 6) upon pupation of larvae. If no growth occurred in the first case, i.e., around the pupae, the medium showed no sign of contamination throughout the life-cycle. Bacterial growths visible around the pupae might disappear during the life of the larvae, but usually reappeared when

[7] Paine ('11) showed that yeast cells are highly permeable to alcohol which readily and permanently plasmolyzes them.

the larvae pupated. Apart from visible growths, the sterility of the tube was tested by introducing loops full of the medium on which larvae were working or had pupated into a sterile tube of one of the following media; potato agar, banana agar, Pasteur's agar, nutrient gelatine, nutrient bouillon and yeast agar. One or all of these media were used to test the environment of the larvae for the presence of microörganisms (fig. 18). Usually crushed adults, pupae, or larvae were also introduced into the test culture tube. The method of inoculation was by stab or streak; in the former case semianaërobes could develop. Banana agar was used most often, as it more nearly resembles the natural environment of the fly and its associated organisms and also can support vigorous growths of a large flora.[8]

TABLE 3

NUMBER OF INOCULATIONS	TIME OF EXPOSURE TO 85 PER CENT ALCOHOL	NUMBER OF CONTAMINATIONS
	min.	
10	0	10
2	1	2
2	5	0
2	10	0
2	15	0
2	20	0

Smears of the media were examined after staining in the usual manner with eosin or Loeffler's methylene blue. This examination was made with a 1.6 mm. Zeiss objective. Fresh smears were examined, before staining, with dark and light field illumination.

B. Food of Drosophila. *a.* Growth of sterile larvae on sterile fruit. From the foregoing experiments it is clear that yeast is always present in the habitat of Drosophila larvae and is usually imported into sterile media on the body of the adult or pupa. Pupae and eggs do not contain living yeasts and any yeasts on the external body surface can be killed by alcohol.

[8] For example, Cocobacillus acridiorum, Bacillus prodigiosus, B. coli, B. aceti, Streptococcus dispar, Saccharomycetes cerevisiae, S. ellipsoideus, S. anomalus, Penicilium glaucum, Rhizopus nigricans, Aspergillus, Fusarium, Gliocladium, etc.

If sterile pupae are placed on a sterile medium of banana agar and protected from contamination, the adults emerge and oviposit, but the larvae that hatch develop very slowly and finally die before pupating. The great difference in rate of growth between sterile and non-sterile larvae on the same food is shown in figure 2. In cultures A 10, 11, 12, amd 17 living yeast cells were present and the larvae grew at a normal rate, reaching the full length of 8 mm. in eleven to twenty-six days when pupation took place. In cultures[9] A 18 and 25, on the other hand, the sterile larvae reached a size of only 3 mm. after twenty-eight to forty-

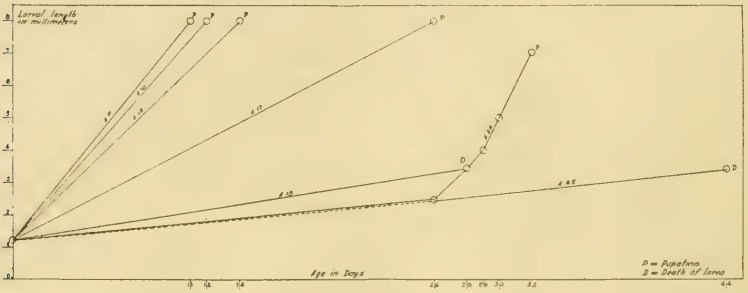


Fig. 2 Larval growth on banana agar. A 10, 11, 12, 17, growth in cultures infected with living yeasts; A 18, 24, 25, slow growth of larvae in sterile cultures; A 24, infected with living yeast on twenty-sixth day, causing an increase in growth.

four days, when they died. In culture A 24 the sterile larvae reached a length of 2 mm. in twenty-six days when the medium

[9] The size of the larvae on different media was determined by placing the tubes and a millimeter scale on the stage of a binocular microscope and measuring the length of five to ten of the larger specimens while 'crawling' at full length. The larger specimens were selected for measurement because, although female adults were allowed to oviposit for only one day, the eggs showed considerable variability from one to three days in their date of hatching, depending on the readiness with which the female oviposited on the medium.

The cultures were kept in a steam-heated room in which the maximum temperature for the entire period of experimentation varied between 96° and 71°F. and the minimum between 73° and 56°F. As compared experiments were run parallel in time, the error due to temperature differences should not be great.

It should be kept in mind that each point on a curve of growth is the average of the whole culture of larvae, i.e., usually twenty or more individuals, thus a single curve has considerable weight.

was inoculated with living yeast. This caused a rapid increase in size and ended in pupation six days later.

The acceleration which takes place on infecting a sterile medium with living yeasts indicates that the alcoholic treatment in sterilizing the pupae does not cause the decrease in the rate of growth of the sterile larvae. Other cases of acceleration which occurred due to accidental contamination of a sterile medium quite often bore out this conclusion.

Therefore, it is certain that sterile larvae grow more slowly than non-sterile larvae on sterile food, and that the rate of growth can be increased by infecting the medium with the living yeast.

b. Is fruit the food for Drosophila larvae or merely the substratum for yeast cells? As sterile larvae grow so slowly and do not pupate in sterile fruit, but develop normally if it is infected with living yeasts, the question arises as to the true position of the fruit in the ecology the insect. By using a medium containing the inorganic salts and the sugars and ammonium tartrate necessary for yeast growth, the starch, oils, fats, proteins, and other substances of the fruit were eliminated from the experiment.

The composition of the medium was as follows:

Agar-agar	4.0 grams	K_2HPO_4	0.165 grams
Grape-sugar	16.5 grams	$MgSO_4$	0.165 grams
Cane-sugar	16.5 grams	H_2O	200 cc.
Ammonium tartrate	3.3 grams		

Sterile larvae lived only five days on this sterile medium and showed no increase in size; but if the medium was infected with living yeasts, the larvae grew at a normal rate, reaching their maximum size in ten days, and pupated normally. The adults which emerged from these pupae were sexually fertile and of large size. Thus, in the presence of living yeast, Drosophila larvae grow normally in a synthetic nutrient medium for yeast with ammonium tartrate as the only supply of nitrogen. Therefore the simplest nutrient medium for yeast if infected with living yeasts is equavalent to fermenting fruit in the ecology of Drosophila larvae.

The nutrient medium for yeast in itself is not an adequate substitute for sterile fruit, as Drosophila larvae live longer on the latter, e.g.,

Medium	*Increase in Length*	*Longevity*
Sterile banana agar	1.8 mm.	26 to 44 days
Sterile yeast nutrient medium	0 mm.	5 days

Therefore sterile fruit has greater food value for sterile larvae then the simplest 'nutrient medium for yeast.' Fruit is mainly the nutrient substratum for yeast cells, but has some food value for Drosophila larvae.

c. Are products of fermentation essential food requirements of Drosophila larvae? In the preceding experiments living yeast cells had an opportunity to develop and form products of fermentation in the media. As these products may have food value for the larvae, the essential difference between a septic and a sterile food might be the absence of these substances. If this were the case, the larvae would be dependent on yeast not as a food, but as a chemical agent.

By boiling yeast before adding it to yeast nutrient agar, the formation of fermentation by-products was prevented. Fleishmann's bread yeast was used for this purpose and 6 grams were added to every 100 cc. of yeast nutrient agar. On this medium sterile larvae grew at a normal rate, reaching their full size in ten days and pupating normally. This proves that Drosophila larvae grow normally on dead yeast in the absence of any by-products of fermentation.

d. Is yeast a complete food for Drosophila larvae? In the media used thus far various substances besides yeast were present. To eliminate these and determine whether or not yeast alone is a complete food for Drosophila larvae, media were made up of Fleishmann's compressed bread yeast, water, and agar-agar.[10] It was found that sterile larvae on a medium of 6 grams yeast per 100 cc. water grew as rapidly as non-sterile larvae and many times faster than sterile larvae on banana. In figure 3 cultures W 3, 4, and 5 show that larvae on dead yeast grew to maximum

[10] Sterile larvae live a maximum of five days on sterile $1\frac{1}{2}$ per cent agar and water medium, showing no increase in size.

size in four or five days, while larvae on sterile banana (A) did not reach their maximum size in twenty-eight days.

The minimum requirement of yeast was found by the use of media consisting of 1, 2, 3, 4, 6, 9, 12, and 24 grams of yeast, respectively, separated in 100 cc. of water and thickened with powdered agar-agar. On sterile 1 per cent yeast the larvae grew very slowly for twenty days, dying at a length of 4 mm. without pupating. On sterile 2 per cent yeast larvae pupated when 5.5 mm. in length, reaching this size on the 10th day. On 3, 4, 6, 9, and 12 per cent yeast media the results were much alike, the larvae

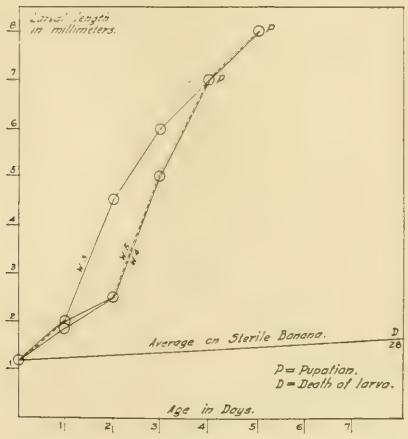


Fig. 3 Larval growth on dead yeast. W 3, 4, 5, show rapid growth on dead yeast; A, shows slow growth on sterile banana.

reaching a size of 7.5 to 8 mm. in length on the third, fourth, or fifth day and pupating before the eighth day. On 24 per cent yeast the larvae often reached a length of 6.5 mm. on the first or second and pupated before the sixth day.

Records of the growth of cultures of larvae, on yeast media of different strengths, follow in table 4 and the mean larval periods are included in table 5. In figure 4, curve 1 shows the rapid growth on 24 per cent yeast; curves 2 and 3, the maximum and minimum rates of growth on 3 to 12 per cent yeast, and curves 7, and 9, the slow growth on 2 and 1 per cent yeast.

TABLE 4

Age of larvae in days

MEDIA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	20	26	27	28
	Length in millimeters																			
Hot aqueous unfermented banana sol.....1		2	3				4				4.5			5		5.5	5.5	5.5	6	P
Campestris.....1				2.5	2.5		5	6				P								
1 per cent yeast.....2		2			2	2.5	3	3	3	3	3		3				4			
2 per cent yeast.....0			2	2.5	2.5	2.5	3.5	5.5	5.5	5.5		P								
3 per cent yeast.....1	2	3		8	8			P												
3 per cent yeast.....2		5.5	6	7		P														
3 per cent yeast.....3		3	5	8			P													
4 per cent yeast.....2	2	3.5	6.5	7	7.5		P													
4 per cent yeast.....3	2	3	6	7	7			P												
6 per cent yeast.....1	2.5	4		7.5	P															
6 per cent yeast.....2		5	6.5	8	8	P														
6 per cent yeast.....4	2	3.5	6	7	8		P													
12 per cent yeast.....2		6	8			P														
12 per cent yeast.....3	2	4.5	6	7	8		P													
12 per cent yeast.....4	1.8	3	5	7	7		P													
12 per cent yeast.....5	2	2	5	7	8			P												
24 per cent yeast.....1	6.5	7	7.5			P														

TABLE 5

MEDIA	NO. PUPAE	MEAN LARVAL PERIOD	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY	NO. ADULTS	MEAN PUPAL PERIOD	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY
Hot aqueous sol. unfermented	8	20.			3	5.		
banana	4	28.75	1.29	4.5	9	5.5		
Banana mash.....1	1	11.0			0			
Banana mash..... 4	2	13.0						
Agaricus campestris.....1	14	12.43	1.89	15.2	14	3.85	0.6221	16.1
1 per cent yeast.....2	0	20.						
2 per cent yeast.....1	10	11.4	1.42	12.4				
3 per cent yeast.....1	29	7.14	0.27	3.78	29	4.75	0.2318	4.8
3 per cent yeast.....2	10	6.00	0.14	2.03				
3 per cent yeast.....3	50	6.32	1.93	30.5	40	4.33	0.0409	0.9
4 per cent yeast.....1	28	13.05[1]	1.55	11.1				
4 per cent yeast.....2	36	6.47	1.70	26.0	33	6.63	11.66	25.0
4 per cent yeast.....3	40	7.20	1.208	14.00	30	5.58	2.89	43.59
6 per cent yeast.....1	17	5.00	0.0	0.0	20	4.0	0.0	0.0
6 per cent yeast.....2	21	6.0	0.308	5.1	20	4.4	0.154	3.5
6 per cent yeast.....4	61	6.46	0.157	2.4	60	4.37	0.2454	5.6
12 per cent yeast.....2	40	5.8	0.5	8.8	40	5.45	1.07	19.6
12 per cent yeast.....3	51	7.0	0.713	10.2	51	4.46	0.6361	14.2
12 per cent yeast.....4	17	6.3	0.525	8.3	17	4.17	0.41	9.8
12 per cent yeast.....5	50	7.8	1.095	14.0	25	3.68	1.2	32.6
24 per cent yeast.....1	60	5.21	1.61	30.9	60	5.44	0.527	9.6
24 per cent yeast.....2	12	4.83	0.188	3.8	12	3.93	2.02	56.0
3 per cent yeast.....average	89	6.55			69	4.50		
4 per cent yeast.....average	76	6.85			66	5.85		
6 per cent yeast.....average	79	6.11			100	4.30		
12 per cent yeast.....average	158	6.87			133	4.07		
24 per cent yeast.....average	72	5.14			72	5.18		

[1] F_1 from adults reared on Agaricus campestris.

These experiments show that dead yeast is an adequate food for Drosophila larvae when in a concentration of 2 per cent or more.

e. Can Drosophila larvae complete their growth on any vegetable food other than yeast? Bacteria and fungi other than yeasts appear to have some food value for Drosophila larvae, as in microscopic examinations of the digestive tract bacteria often form the bulk of the contents. The following experiments show

that these microörganisms are not as valuable to the insect as yeast cells. A few larvae were reared on vinegar-plant agar, pupating on the sixth day. On manure agar growth was slower and pupation took place on the fifteenth day. On lactic acid bacillus and on Rhizopus nigricans agar no growth took place, but the larvae died in three to five days. On plain agar infected with a semianaërobic bacterium a few larvae pupated after twenty-six days. Therefore yeasts are a more complete food for Drosophila larvae then other bacteria or fungi.

I have already shown that fruit (banana) is of some food value for Drosophila larvae, as it will keep the insects alive for periods

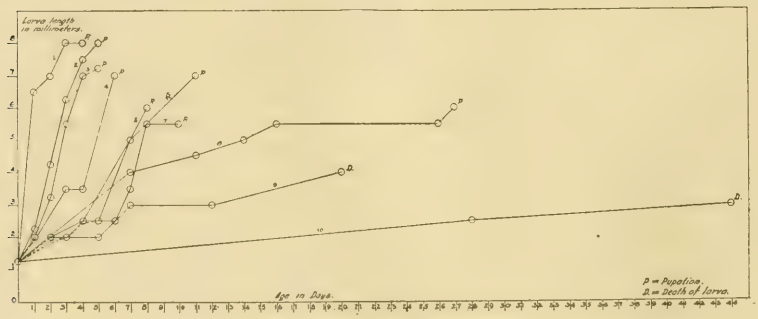


Fig. 4 Larval growth on various media. 1, 24 per cent yeast; 2, maximum 3 to 12 per cent yeast; 3, minimum 3 to 12 per cent yeast; 4, vinegar plant; 5, mushroom; 6, yeast nucleoprotein, sugars, and salts; 7, 2 per cent yeast; 8, hot aqueous extract of banana; 9, 1 per cent yeast; 10, cold aqueous extract of banana.

of twenty-eight to forty-four days and permit them to increase in size to a limited extent.

The activity of the larvae and analysis of the banana indicate that the insect is abundantly supplied with carbohydrates (20 per cent sugar in ripe fruit). The protein content, on the other hand, is relatively low (1 per cent) and is probably deficient.

The long life of the larvae on sterile banana with the accompanying slow increase in size, indicates that all the food elements required for maintenance and repair of tissues are present, but the protein content is either too small or lacking or deficient in some amino-acid necessary for growth. There is also the possibility

that some vitamine may be absent or may have been destroyed by the high temperatures of the autoclave.

Some light is thrown on these questions by a comparison of the rates of growth of larvae on banana media which have been more highly concentrated by partial desiccation or by extraction with hot water. The growth of insects on these media is shown in table 6 and figure 5.

On sterile food consisting of mashed whole bananas, especially when they have dried out slightly and are thus concentrated, an occasional small pupa is formed. On a hot aqueous extract of banana a larger number is formed from which small adults emerge.

TABLE 6

MEDIA	LARVAL PERIOD (20 OR MORE LARVAE IN EACH CASE)	NO. PUPAE	NO. ADULTS	CONTAMINATION
Banana mash 1	11 days	1	0	−
Banana mash 2	7 days	16	16	+
Banana mash 3	22 days	1	1	−
Banana mash (slightly shrunken) 4	13 days	2	2	−
Cold aqueous extract of fermented banana	28 days (Average)	0	0	−
	12 days (Average)	73	70	+
Cold aqueous extract of unfermented banana	25 days (Average)	0	0	−
	13 days (Average)	90	85	+
Hot aqueous extract of unfermented banana	20 days	8	8	−
	29 days	9	9	−
	9 days (Average)	68	66	+

These are potentially fertile, for when fed with yeast, the females deposit fertile eggs from which normal larvae emerge. This will be discussed below at greater length. On cold aqueous extracts of unfermented banana, no larvae pupate.

These results show that concentrated banana permits complete and more rapid growth of larvae. Therefore, the fruit is not entirely lacking in any amino-acid necessary for growth nor is any vitamine absent or destroyed by sterilization. It appears, moreover, that concentrated banana forms a complete food for Drosophila larvae, i.e., the protein deficiency of fruit is quantitative rather than qualitative.

As Agaricus campestris has a protein content of 3.5 per cent as compared with the 1 per cent protein content of banana, this fungus was used as a food for larvae. Drosophila females oviposited readily on a medium of powdered Agaricus campestris, water, and agar. The sterile larvae grew rapidly to a size of 6 mm. (the maximum size on yeast is 8 mm.) and pupated after an average of 12.43 days. The curves of growth are shown in figure 6 and a typical record in tables 4 and 5. The adults that emerged from these pupae were sexually fertile, but were quite small. A generation of larvae reared from some of these adults grew more slowly than normal on 4 per

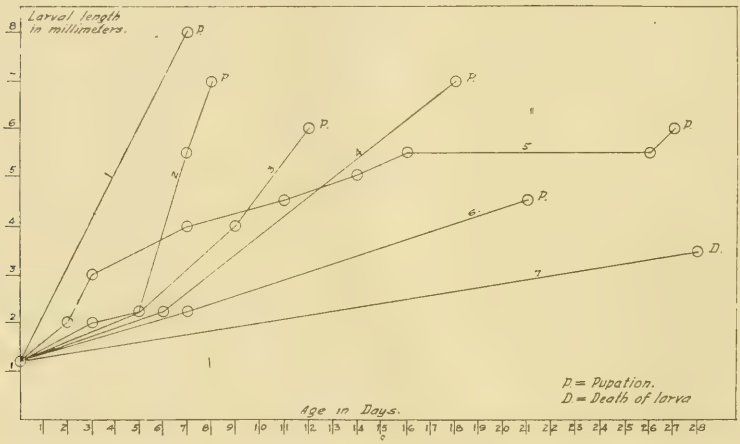


Fig. 5 Larval growth on banana. 1, mashed whole banana infected with living yeast; 2, 3, 4, sterile mashed whole banana; 5, 6, hot aqueous extract of banana; 7, cold aqueous extract of banana.

cent yeast, requiring thirteen days instead of seven days to reach maturity (fig. 6, curve 2, and table 5). Sexually fertile adults can be reared from larvae fed on mushrooms, but such adults are undersized. Agaricus campestris meets more nearly the food requirement of larvae than banana; this may be due to the higher percentage protein content of the mushroom or to a relatively higher content of certain necessary amino-acids.

f. Is yeast a more adequate food than fruit because of its high protein content? The slow growth and small size of larvae and their failure to pupate when reared on sterile fruit are typical

symptoms of protein deficiency. This deficiency is quantitative rather than qualitative, because more normal growth of the larvae is permitted when the fruit is concentrated.

As these symptoms of malnutrition are not shown in larvae reared on yeast, we would expect to find a high protein content of adequate components in this food. This assumption is correct, for Atwater and Bryant ('02) have found by analysis that the percentage protein content of yeast is 11 per cent. This is higher than the percentage occurring in any fruit or in Agaricus campestris, and Meisenheimer ('05) has shown that most monoamino-acids occur in yeast protein.

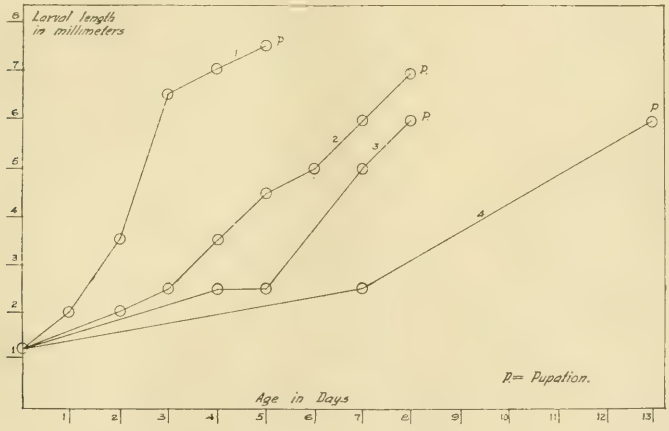


Fig. 6 Larval growth on mushroom. 3, 4, mushroom; 1, normal growth on 4 per cent yeast; 2, growth of larvae of mushroom-fed adults on 4 per cent yeast.

Is the rapid growth of larvae on yeast due to this high protein content or to other substances present in the yeast cell, such as glycogen, fat, gums, hemicelluloses, etc.? By extracting the yeast nucleoprotein and making media with known sugars and salts, this problem was solved. The method employed to extract the yeast nucleoprotein and to make the media is described in a foot-note below.[11]

[11] The pure yeast nucleoprotein was obtained in the usual manner (Hawk, '16), as follows: the cells in 4 pounds of baker's yeast (Fleishmann's bottom bread

The growth of larvae on yeast nucleoprotein media is shown in figure 7 and table 7. Table 7 shows that larvae do not grow on nucleoprotein in the absence of Pasteur's solution, but, if these salts and sugars are added, do grow rapidly and form large numbers of normal pupae from which normal highly fertile adults emerge.

yeast) were killed by ether and broken open by grinding in a mortar with a quantity of pure white diatomaceous earth, adding enough water to keep the mass in a sticky, smooth condition. The grinding was continued till examination with a 1.6-mm. objective showed that many of the yeast cells had been cut into irregular rectangles. The yeast was then poured with the addition of 0.4 per cent NaOH solution into a large bottle and 8000 cc. of the alkali were added. About 40 cc. of chloroform were then mixed with the solution to prevent the development of bacteria. The contents of the bottle were thoroughly agitated several times a day. After forty-eight hours the supernatant fluid was poured off, leaving a great part of the yeast and diatomaceous earth in the bottle. This fluid was centrifuged in a large-sized electric centrifuge with a capacity of four 250 cc. bottles. Each liter was run for twenty minutes (fifteen minutes at maximum speed) and the supernatant fluid carefully poured off. This fluid was examined for yeast cells with a 1.6-mm objective and showed an entire absence of them. After all the yeast cells had been removed in this manner, the liquid had a clear opalescent color and proved to be rich in nucleoprotein. This was precipitated in great white floccules by adding 10 per cent HCl in drops. The precipitate dissolved in the alkaline or neutral solution, but remained in the slightly acid solution in which the largest amount was formed. The precipitate was separated pure from the solution by centrifuging and washing with acid alcohol and neutral alcohol in which it was insoluble. The white precipitate was then dried over H_2SO_4, forming a white powder. The remaining fluid was neutralized with N/10 NaOH and dialyzed for five days in running water, keeping the surface covered with toluol. No precipitate was formed. The neutral, salt-free solution was then heated to boiling and acidified with a drop of HCl or acetic aid and also acidified and then boiled. No marked precipitate was formed. Half saturation, complete saturation with $(NH_2)SO_4$ when hot or cold and saturation with NaCl and with picric acid failed to bring down any precipitate. A heavy precipitate which appeared to be a peptone decomposition product of the nucleoprotein was produced upon the addition of phosphomolybdic acid.

The nucleoprotein was ground in a mortar and then made into media as follows: 1) Nucleoprotein moistened with tap-water was placed in test-tubes and sterilized in the autoclave; 2) nucleoprotein moistened with Pasteur's nutrient solution (grape-sugar, cane-sugar, ammonium tartrate, $MgSO_4$, K_2HPO_4, H_2O) in test-tubes and sterilized; 3) nucleoprotein and 1.5 per cent agar-agar tap-water solution autoclaved and mixed aseptically in sterile test-tubes, and, 4) nucleoprotein. Pasteur's nutrient solution and 1.5 per cent agar solution autoclaved and mixed aseptically in sterile test-tubes. If mixture is made before autoclaving the protein adsorbs (?) the agar and on cooling jellation does not take place.

Adults placed on media from which sugars were absent died after one to four days, whereas those placed on media containing Pasteur's solution lived for a much longer time. The larvae on the nucleoprotein alone live for several days, but do not increase in size and are not very active. It may be that a sweet taste is necessary to stimulate them to take food or it may be that carbohydrates are necessary to furnish energy "fuel." The larvae on nucleoprotein and carbohydrates grow slowly at first, but quite rapidly after reaching a length of 3 to 4 mm. This may be due to the rather large size of the nucleoprotein crystals or to the depth

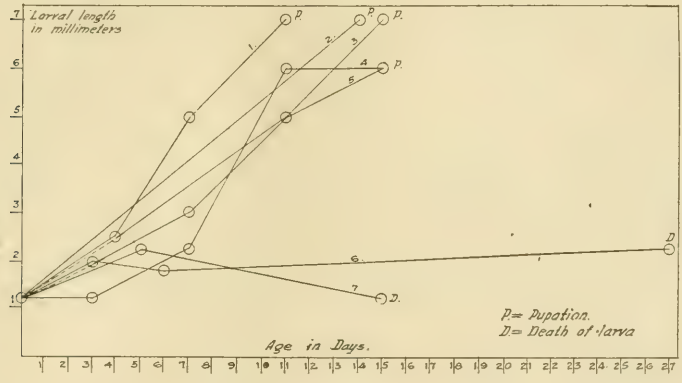


Fig. 7 Larval growth on yeast nucleoprotein. 1, 2, 3, 4, 5, yeast nucleoprotein, sugars, and salts; 6, 7, yeast nucleoprotein and tap-water.

to which they sink in the agar. In figure 7 are shown the curves of growth on these media. Curves 1 to 5 show the rapid growth on yeast nucleoprotein, sugars, and salts and curves 6 and 7 show the slow growth or diminution on nucleoprotein alone. One curve of growth on yeast nucleoprotein and sugar, etc., given (fig. 4, curve 6) in the same figure with curves for yeast media, shows that larvae grow more rapidly on yeast nucleoprotein and sugar than on 2 per cent but less rapidly than on 3 per cent yeast. It must be remembered that mechanical questions of ingestion and the question of taste or olfactory preference may largely affect the amount of material eaten and therefore the rate of growth.

TABLE 7

MEDIA	NO. DAYS ADULT LIVED ON MEDIUM	LARVAL PERIOD	NO. PUPAE	NO. ADULTS	CONTAMINATION
Nucleoprotein + tap water No. 1.....	2	6	3	3	+
Nucleoprotein + tap water No. 2.....	2	5	4	4	+
Nucleoprotein + tap water No. 3.....		12d	0	0	—
Nucleoprotein + tap water No. 4.....		10d	0	0	—
Nucleoprotein + Pasteur's sol. No. 1.		10	1*	0	—
Nucleoprotein + Pasteur's sol. No. 2.....		5	0*	0	—
Nucleoprotein + Pasteur's sol. + agar No. 1.	5+	22	3+	1+	—
Nucleoprotein + Pasteur's sol. + agar No. 2.	6+	16	80+	80+	—
Nucleoprotein + Pasteur's sol. + agar No. 3.		21	10+	?	—
Nucleoprotein + Pasteur's sol. + agar No. 4.	7	12	30	30	—
Nucleoprotein + Pasteur's sol. + agar No. 5.		14	3	0	—
Nucleoprotein + tap water + agar No. 1.....		5d	0	0	—
Nucleoprotein + tap water + agar No. 2.....		21d	0	0	—
Nucleoprotein + tap water + agar No. 3.....	3	16d	0	0	—
Nucleoprotein + tap water + agar No. 4.....		5d	0	0	—
Nucleoprotein + tap water + agar No. 5.....	1	5d	0	0	—
Nucleoprotein + tap water + agar No. 6.....	2.5	5d	0	0	—
Nucleoprotein + tap water + agar No. 7.....	4	0x	0	0	—

x No eggs laid.
d indicates death.
* Media dried.

Hence the objection that there is no exact mathematical correlation between the rates of growth and the protein concentration is not irrefutable evidence against the view that primarily such a relationship exists. For example, some farm animals will lose weight on a soy-bean diet of high protein value, but of a taste they do not like.

Since Drosophila grows normally, pupates in large numbers, and develops into fertile adults of good size, it appears that a medium of yeast nucleoprotein, sugars, and inorganic salts is a complete food for this insect. It has already been proved by experiment (p. 13) that larvae do not grow on sugars and inorganic salts alone, so that the nucleoprotein is the substance which, if added, makes the medium equivalent to yeast cells as food for

the insect. As the sugars and inorganic salts are abundantly present in fruit[12] and the addition of nucleoprotein of yeast is sufficient to make the synthetic medium a complete diet for Drosophila, it can be said that yeast is a more adequate food than fruit because of its high protein content.

g. Conclusions. 1. Insects can be conveniently reared in a solid agar medium.

2. Larvae prevent the development of molds on the medium, but are always associated with living yeasts.

3. For genetical work fermented banana agar or Pasteur's culture fluid agar is most convenient.

4. Living yeasts are not present in the egg or pupa.

5. The exterior of pupae can be sterilized by washing in 85 percent alcohol for twenty minutes. Yeast cells are more readily killed by this treatment than molds.

6. Banana agar is a good culture medium for fungi.

7. Sterile larvae grow more slowly than non-sterile larvae on sterile fruit; the rate of growth can be increased by infecting the medium with living yeasts.

8. The alcoholic treatment in sterilizing pupae is not the cause of the slow growth of larvae on sterile food.

9. The simplest nutrient medium for yeast, if infected with living yeast, is equivalent to fermenting fruit in the ecology of larvae.

10. Sterile fruit has greater food value for larvae than "sterile nutrient medium for yeast."

[12] Atwater and Bryant ('06) give the following analysis of the edible portion of banana:

	Water per cent	*Protein per cent*	*Fat per cent*	*Total carbohydrate per cent*	*Ash per cent*	*Fuel value per lb.*
Minimum	66.3	1.0	0.0	16.3	0.5	330
Maximum	81.6	1.6	1.4	29.8	1.1	640
Average	75.3	1.3	0.6	22.0	0.8	460

Prescott ('17) gives this analysis of banana ash:

	per cent		*per cent*		*per cent*
Silica	2.19	Phosphoric acid	7.68	Potash	43.55
Lime	1.82	Magnesia	6.45	Sulphur trioxide	3.26
Iron oxide	0.18	Soda	15.11	Chlorine	7.23

11. Fruit is mainly the nutrient substratum for yeast cells, but has some food value for larvae.

12. By-products of fermentation are not necessary for larvae.

13. Dead yeast is an adequate food for larvae when in a concentration of 2 per cent or more.

14. Yeast is a more complete food for larvae than other fungi.

15. Concentration of banana by hot-water extraction or drying makes it an adequate food for larvae.

16. The protein deficiency of fruit is quantitative rather than qualitative.

17. Agaricus campestris meets more nearly the food requirements of larvae than banana.

18. Yeast nucleoprotein, sugars, and salts are an adequate food for larvae.

19. Yeast is a more adequate food than fruit because of its higher protein content.

C. Discussion. a. Effect of food on larval, pupal and adult life. On page 15 and in table 5 and figure 4 it has already been shown that the concentration of yeast affects the length of the larval period. This effect can be seen more clearly if we plot the larval period on the axis of ordinates (vertical), of a graph, and the number of grams of yeast per 100 cc. of water, in the media, on the axis of abscissae (horizontal). A curve drawn through the points established represents the effect of the concentration of yeast upon larval life. This curve is drawn in figure 8. It changes its direction very suddenly at a point between 2 and 3 per cent of yeast, going up from a larval life of 6.55 days on 3 per cent to a period of 11.40 days on 2 per cent yeast. If we continue the curve in the same direction we approximate a period of twenty days representing the larval life (which ends in death) on 1 per cent yeast medium. This great change in the direction of the curve indicates that there is a definite concentration (2 per cent) of yeast necessary for the completion of the larval period. Between yeast concentrations 2 per cent and 3 per cent there is a difference in the larval period of 4.95 days, whereas between concentrations 3 per cent and 12 per cent there

is only a variation of 0.76 day and between 3 per cent and 24 per cent—a difference of 1.11 days. It appears that the normal condition for larval growth is in a medium of yeast concentration between 6 to 24 grams per 100 cc. This is shown in the size of pupae in table 8.

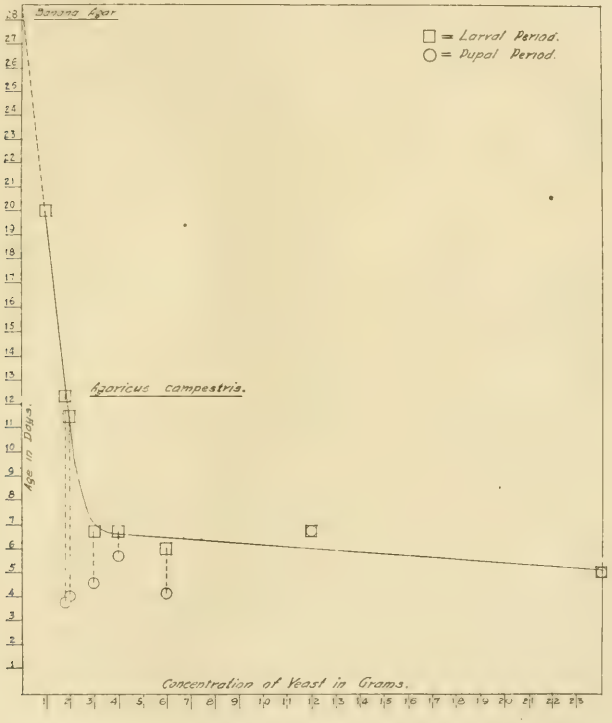


Fig. 8 The effect of the concentration of yeast on the length of the larval and pupal periods.

A concentration of 1 per cent of yeast appears to be sufficient to furnish the energy, repair, and some growth requirements of the larvae for a considerable period without furnishing quite enough food to allow the necessary growth changes or storage preliminary to pupation. The larval period must be considered as a nutrition unit. In the case of larvae on 2 per cent yeast, the insect obtains in 11.4 days sufficient food material to give

TABLE 8

Effect of larval food on size of pupae

MEDIA	LENGTH OF PUPAE	ADULTS
	mm.	
Banana mash	2.0–2.5	
2 per cent yeast	2.5–3.0	
Hot aqueous sol. unfermented banana	3.5–4.0	
Agaricus campestris	3.5–4.5	Undersized
3 per cent yeast	4.0–5.5	
4 per cent yeast	5.0–5.5	
6 per cent yeast	5.5–6.0	
12 per cent yeast	5.0–6.0	Normal size
24 per cent yeast	5.0–6.0	

the energy, wear and tear, growth and storage requirements. On 3 per cent yeast the larval period approached normal at the expense of the reserve stuffs in the pupa, for the latter is undersized. This is also true of 4 per cent yeast. On 24 per cent the larvae usually reach a size of 6.5 mm. in length on the first day and are therefore three days ahead of all larvae on media of 3 per cent yeast; still pupation occurs only 1.41 days earlier. Apparently there is a certain periodicity in the larval life, since there is a tendency for the larva to pupate after a certain length of time whether it reaches the maximum size before this period or is still undersized. The probable explanation of this phenomenon is that certain changes go on in the larva, since a metamorphosis of the nervous system and digestive glands is known to take place during this period, at a definite rate if the minimum necessary food substances are available.[13]

The pupal periods (table 5) of Drosophila, fed during larval life on different concentrations of yeast, are also plotted in figure 6. The figures show that there is no consistent variation between the pupal period of larvae which lived for a long or a short

[13] Mendel and Judson ('16) studied the proportional weights of skeletons of retarded rats and found that the skeleton grows at a normal rate in retarded individuals. On normal food the growth of retarded individuals is accelerated, but that of the skeleton is retarded till equilibrium between tissue and skeleton weight is established.

period before transforming. Therefore, the pupal period is not correlated with the length of the larval life, i.e., it has, also, a fixed periodicity.

The growth of insect larvae may be retarded on sterile fruit and then greatly accelerated by adding living yeast cells to the food. Three curves showing this effect are drawn in figure 9 and curve A 24, figure 2 has already been referred to (p. 13). The insect can be maintained for a long period of time on a minimum of protein (banana) at the same size or slowly increasing

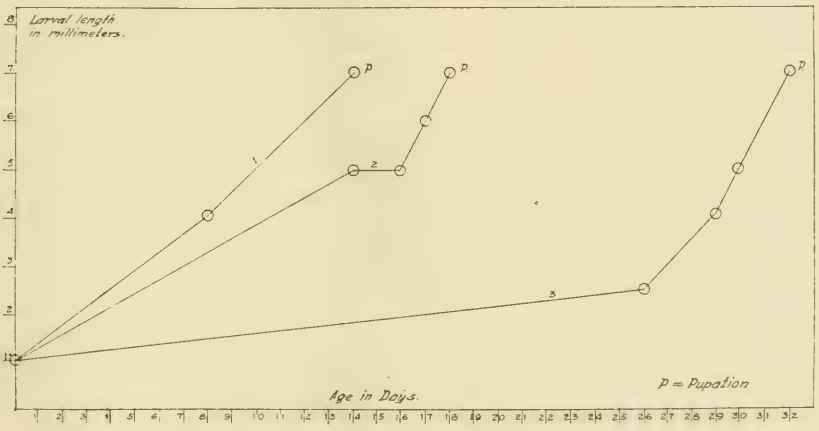


Fig. 9 The growth of retarded larvae. 1, larvae from hot aqueous extract of banana placed on 4 per cent yeast on eighth day; 2, larvae from hot aqueous extract of banana placed on 9 per cent yeast on fourteenth day; 3, larvae from cold aqueous extract of banana, living yeast introduced on twenty-sixth day.

size, after which it may be made to develop to normal size by placing on an adequate diet (6 per cent yeast). Except in one case (fig. 9, curve 3) the acceleration in the growth of retarded individuals was not above the normal rate of larvae on an adequate diet. This may be due to the fact that the length of the larva is a poor criterion of the extent of its development and that units of one day are not accurate for an animal with a normal larval period of about six days.

In this connection it is of interest that Osborne and Mendel ('14) have shown that rats can be kept for a long period at the

TABLE 9

Lengthening of life cycle by retarding larvae

AGAR MEDIA	EGG PERIOD	LARVAL PERIOD	PUPAL PERIOD	LIFE-CYCLE IN DAYS
	days	*days*	*days*	
24 per cent yeast	1–3	5.14	5.18	11.32–13.32
12 per cent yeast	1–3	6.87	4.07	11.94–13.94
6 per cent yeast	1–3	6.11	4.30	11.41–13.41
4 per cent yeast	1–3	6.85	5.85	13.7 –15.7
3 per cent yeast	1–3	6.55	4.50	12.5 –14.5
Hot aqueous extract banana	1–3	28.75	5.5	35.25–37.25
		20.00	5.0	26. –28.0
Retarded on cold extract banana. Accelerated on 26th day with living yeast	1–3	32.0	5.0	38. –40.0

same body weight by underfeeding of protein or by feeding on proteins lacking in the amino-acids necessary for growth. In this way the "menopause was postponed long beyond the age at which it usually appears." The capacity to grow after adult age is not lost if the rats have been retarded ('15), since after being stunted for 100 days beyond the normal growth period, they may reach full size when put on an adequate diet. It "appears as if the preliminary stunting period lengthened the total span of their life" ('17). In March, 1916, these investigators produced evidence from their experiments that "after periods of suppression of growth, even without loss of body weight, growth may proceed at an exaggerated rate for a considerable period."

Larvae which have been retarded in their growth by an inadequate diet and then given the proper amount of yeast food develop into normal pupae and adults. The pupal period remains the same for larvae living twenty-eight to thirty-two days as for larvae that have lived five to seven days. This is shown clearly in figure 8 and table 5. Thus the total span of the life-cycle could be increased from eleven days to forty days or more by retarding the rate of larval growth. This is shown in table 9.

b. Sugar requirement of adults and larvae. The preceding experiments on synthetic media show that both adults and larvae

TABLE 10

AGAR MEDIUM	LARVAL LIFE IN DAYS	ADULT LIFE IN DAYS
Nucleoprotein + sugars + salts + water..	10 to 21 (pupate)	5 to 7+
Nucleoprotein + salts + water.....	5 to 21 (die)	1 to 4
Sugars + salts + water.....	5–(die)	5 to 10+

require sugar as food. On nucleoprotein and sugars larvae live ten to twenty-one days, grow to full size and pupate normally and adults live more than five days. On nucleoprotein and water, however, the larvae live five to twenty-one days without marked increase in size, but all adults die in one to four days. These results are shown in table 7 and figure 7. On plain sugar and inorganic salts, adults live five to ten days, while larvae die in less than five days without increasing in size. The facts are summed up in table 10.

These results show that larvae require sugars for successful pupation, but live longer on pure[14] nucleoprotein than on pure sugar. Adults, on the contrary, live longer on pure sugar than on nucleoprotein alone.

From general observations it appears that adults oviposit more readily on sugar agar than on nuleoprotein agar, but deposit most eggs and over the longest period on nucleoprotein-sugar agar. Therefore it would seem that sugars stimulate oviposition and nucleoprotein increases egg production and that both sugars and proteins are necessary for the normal activities of both larvae and adults.

c. The natural habitat of Drosophila. I have already shown by experiments that Drosophila requires sugars and protein and that these substances can be supplied in a most normal form in yeast cells, so that it is of interest to study the conditions that exist in the natural environment of the insect.

Schultze ('11) has recorded the fly as occurring in many different fermenting and decaying fruits, vegetables and fungi, fermenting tree sap, vinegar, tumors, and animals preserved in formol. Sturtevant ('16) describes a number of new species of

[14] Carbohydrates are formed as decomposition products of nucleoprotein.

Drosophila from decaying fruit, vegetables and fungi, and from feces. Henneberg ('02) suggested that the larvae probably live on the microörganisms present in these media. It is apparent that microörganisms are usually present in great abundance in the larval environment, and from my experiments it appears that they normally serve as food for the insect, since the adults are attracted by the odors of fermentation products (p. 68). Microorganisms, known to occur on the exterior of fruits, probably usually contaminate the substratum before the flies oviposit. Flies assist in establishing a suitable flora by accidentally carrying in the digestive tract or on the minute hairs of the body many yeast cells. This can be seen in cross-sections of the body (figs. 10 and 11) and has previously been shown to be the case on page 8. The yeast growth takes place more rapidly in the presence of larvae, because the latter spread the cells throughout the medium. At first sight, this would appear to throw doubt on the ability of the larvae to digest the yeast cells, but serial sections show clearly the process of disintegration. Figures 11, 12, 13, and 14, are microphotographs of successive sections through the stomadaeum, mesenteron, and proctodaeum showing the process of disintegration which takes place mainly in the middle digestive portion of the tract. However, as in the case of birds which feed upon insect eggs and seeds, many living cells pass through the alimentary canal (page 8).

In nature Drosophila larvae are usually found in a substratum suitable as a nutritive medium for microörganisms and abounding especially in yeasts. In this environment the insect has available as food both the substratum, usually fruit, and the microorganism. But why have the larvae become dependent on the latter? Three reasons immediately come to mind, viz.:

1. As all fruits which are soft enough for Drosophila larvae to live in are always infected with yeast from the air, the larvae would unavoidably ingest fungous cells with the fruit. The nutritive value of the food would soon affect the life-cycle of the insect and bring about a close adaptation to a yeast diet.

2. Larvae feed upon microörganisms and by their constant movements carry the spores throughout the substratum. In

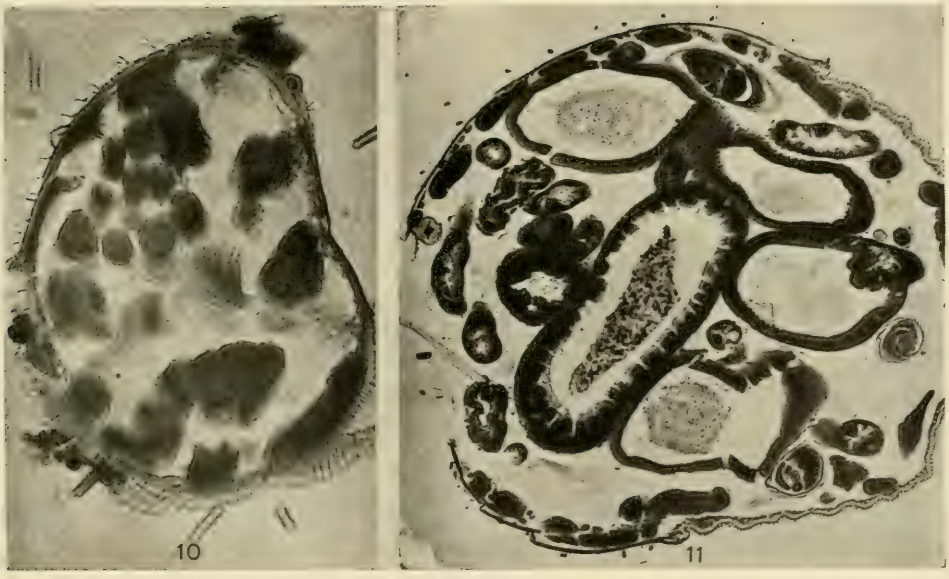
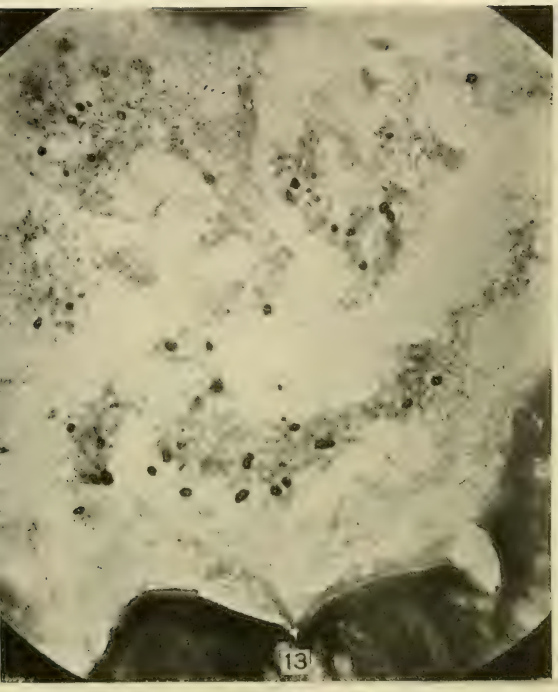


Fig. 10 Microörganisms on exterior of adult Drosophila (section through leg) (p. 32).

Fig. 11 Cross-section through abdomen of adult Drosophila showing ingested microörganisms (p. 32).



Figs. 12, 13, 14 Serial cross-sections through the digestive tract of a Drosophila larva showing the digestion of microörganisms (p. 32).

this manner yeasts often become predominant and prevent the development of destructive molds. These habits of the larvae may have come about through selective killing, for a mortality of 75 per cent was shown in cultures where the activities of the insects were suspended by low temperatures. This high mortality occurred only in cultures contaminated by fungi and was not due to the low temperature itself, but to the uncontrolled growth of the microörganisms.

3. Another explanation may be drawn from a consideration of the relative food value of the substratum and the microörganisms. The fruits or vegetables of the substratum (omitting beans, corn, spinach, etc., which are unlikely to form an appreciable number of breeding places have less than 2 per cent protein and are relatively rich in carbohydrates. Microörganisms, on the other hand, have a protein content of over 10 per cent, but are poor in carbohydrates.[15] This is shown in table 11.

The protein content of yeast cells is readily available, as the enclosing cell membrane has been shown to be closely allied to pectin (Casagrandi). Salkowski ('94) finds that the membrane is composed of two layers, one of which forms on hydrolysis *d*-glucose and the other glucose and mannose. The membranes are readily destroyed by the digestive juices, as yeast is extensively used as protein food for farm animals and even for man (Salomon, '16). The components of the yeast nucleoprotein include almost every known monoamino-acid cleavage product,

[15] Kappes' ('89) analyses of Micrococcus prodigiosus scraped from the surface of solid media gave on an average: Water, 85.5 per cent, and dry matter, 14.5 per cent, the latter having a percentage composition of protein (N × 6.25) of 71.2 per cent, i.e., 10.3 per cent for the whole cell. Nucleoprotein has been separated from cholera, bubonic plague, anthrax, and diphtheria bacilli and from B. pyocyaneum megaterium and Staphylococcus pyogenes aureus (according to Benecke, '12) and from yeasts by Hoppe-Seyler ('71), Kossel ('79), and Stutzer ('82).

Clautrian ('95) showed the presence of glycogen in dried Boletus edulis (20 per cent), Amanita muscaria (14 per cent) and yeast (31 per cent). In 1866 Hoppe-Seyler found 0.25 gram lecithin and 0.44 gram cholesterin in 81 grams of dry yeast, and later Naegli and Loew ('78) found 5 per cent fat (stearic and palmitic acid) in the yeast cell. Yeast gum (mannan) makes up 6 to 7 per cent of yeast (by dry weight), according to Salkowski ('94). Later the following gums were isolated from different yeasts, viz., mucin, dextran, laevulan, mannan, arabin and galactane (Lafar, '03).

TABLE 11

FOOD MATERIAL	WATER	PROTEIN	FAT	CARBOHYDRATES	ASH	AUTHORITY
Fruits	85.9–37.5	1.0–0.2	1.2–0.0	14.4–2.7	0.6–0.1	Atwater ('17)
Vegetables	94.3–44.2	1.8–0.4	0.6–0.1	21.9–2.2	3.2–0.4	Atwater ('17)
		8.65				Stutzer ('82)
Yeast	65.1	11.7	0.4	21.0[1]	1.8	Atwater ('02)
B. prodigiosus	85.45	10.33	0.70		1.75	Kappes ('89)
Putrefactive bacteria	83.42	13.96	1.00		0.78	Nencki and Scheffer ('80)
Mushroom	88.1	3.5	0.4	6.8	1.2	Atwater ('17)

[1] Probably largely starch in the compressed yeast. Glycogen makes up 31 per cent (by dry weight) and gumes 6 per cent of the yeast.

viz., glycocoll, alanine, valine, leucine, proline, phenylalanine, aspartic and glutamic acids, tyrosine, tryptophane, and probably serine and cystine (Meisenheimer, '15), and it is therefore not surprising that it forms, with sugars and salts, a complete food for Drosophila. As the preceding experiments show that Drosophila larvae require more concentrated protein than is present in the substratum, it is apparent that the habits of the insect are for this reason, adapted to the use of the richly protein microorganisms as food.

d. Function of yeast in the ecology of Drosophila. The function of yeast in a Drosophila culture is clearly defined by the following two experiments:

1. Larvae grow slowly on a weak, cold-water extract of aseptic unfermented bananas and remain at about the same size for a period five times the normal life and then die without pupating. If this culture is left open for a few minutes, in such a position as to allow a few fungous spores to fall into the medium, the larvae will increase their rate of growth and pupate in a few days.

2. Larvae on sterile 1 per cent yeast agar grow to a length of 4 mm. in twenty days and die without pupating. If the culture is inoculated with a minute quantity of yeast cells the larval period is only seven days and is followed by pupation. In both cases the yeast cells remove, by adsorption from the medium, the amino-acid molecules in their immediate neighborhood. As this goes on a steady diffusion of amino-acid molecules occurs

towards the place of lowest concentration, and thus the yeast finally adsorbs and builds up into its own protein all the amino-acids of the substratum. The yeast grows at the surface or just below it where it is carried by the larvae and therefore brings within reach of the larvae nitrogen that had been distributed throughout the medium, many parts of which could not be reached. In the experiment with banana agar the yeast not only concentrated the amino-acids of the substratum, but probably synthesized them into more complex molecules; in the second example, the living yeast merely concentrated all amino-acids at the surface of the medium without increasing their complexity. In a synthetic medium of sugars and salts, yeasts would concentrate and synthesize into protein, the ammonia of the substratum.

It has already been shown that concentrated banana permits larvae to pupate, but the rate of growth is not normal. Therefore it is apparent that while the banana is not entirely lacking in the substances necessary for complete growth, it is not as adequate to these demands as yeast or yeast nucleoprotein. Therefore we may conclude that the function of yeast in the ecology of Drosophila larvae is to concentrate at the surface and synthesize the ammonia[16] and aminoacids of the substratum into nucleoprotein, which fills the protein requirements of the larvae.

e. Literature on the food of Drosophila. Valuable contributions to our knowledge of the food relations of microörganisms to insects have been made by Delcourt and Guyénot. These authors reported in 1910 experiments with Drosophila in which they showed that the larvae could be reared on a potato medium free from all microörganisms except yeast or a complex of yeast and acetic-acid bacilli. Microscopical examination showed the yeast cells in the digestive tract in all stages of digestion. This paper was followed by a second in 1913 (a) in which the authors determined whether the insect fed on the products of the yeast's chemical activities or upon the yeast cell itself. In order to obtain this information, it was necessary to operate with larvae

[16] Yeast can also synthesize protein from a urate source of nitrogen.

that were sterile or with which only a single species of microörganism was associated. This was accomplished by means of an ingenious method for the aseptic transfer of adults from one flask to another and by the use of various media adverse to different species of molds and bacteria. This method of sterilizing the larvae is much less direct and requires more time than my method of sterilizing pupae with alcohol. The flies were finally found to be sterile except for the presence of yeast cells and these were eliminated by a rapid transfer of females from bottle to bottle, thus permitting aseptic oviposition in a few cases. The sterile larvae which emerged were then fed on a medium of potato and dead baker's yeast or dead baker's yeast, water, and cotton. The authors at this time made no definite statement about the function of the microörganisms, but left that for later papers. In 1913 (a) Guyénot reported that he had been able to raise fourteen generations of Drosophila in the absence of living organisms. The larvae were reared equally well on potato and living yeast, potato and dead yeast, and on dead yeast alone, but did not grow normally on sterile potato. Guyénot ('13 b) therefore concluded that in nature the larvae nourish themselves principally on living yeast and other microörganisms.

The work of Delcourt and Guyénot was unknown to me until after I arrived at similar conclusions[17] by different methods. The experiments with Drosophila as reported above are therefore in part an independent corroboration of the work of these authors.

Loeb ('15) reared Drosophila on a medium of salts and sugars with ammonium tartrate as the only source of nitrogen and therefore concluded that this insect has as great synthetic power as bacteria. Later ('16) he pointed out that yeasts may have been intermediate in the synthesis of protein, and in a third paper (Loeb and Northrop, '16 b) showed that yeasts serve as food for Drosophila and are required for the growth of the larvae.[18] These authors were unable to isolate the substance in the yeast

[17] My experiments extended over the entire period between May 1, 1916, and June 1, 1917, and were partially published in three papers (see Bibliography).

[18] Eggs were sterilized by washing in 0.1 per cent $HgCl_2$ for six to seven minutes.

on which larval growth depended, but found that the microórganism when extracted with hot alcohol could no longer serve as food for the insect. The addition of those special substances necessary to higher animals did not take the place of the substance extracted from yeast. The insects could not be reared on the normal salts, sugars, and amino-acids or proteins sufficient for higher animals, viz., cane-sugar, $MgSO_4$, NaCl, and $CaCl_2$, with casein, edestin, egg albumin, or a mixture of leucine, alanine, glycine, asparagine, tyrosine, tryptophane, and histidine, or with milk. Twelve successive generations of the flies were raised aseptically on yeast, water, and citric acid. It should also be mentioned that Loeb and Northrop raised aseptic flies on aseptic unfermented banana, but were unable to secure a second generation from them even after feeding the adults on yeast, as both sexes were sexually sterile.

My experiments show that Drosophila can be reared normally on yeast nucleoprotein, sugars, and salts, therefore any 'special substance' required by the larvae must be present in this mixture.

As previously mentioned, I have been able to rear sterile larvae on sterile hot aqueous extract of banana agar and obtain adults which appeared to be sexually sterile, as they did not oviposit on the banana during six days (the usual preoviposition period being twenty-four to forty-eight hours), but when half of the number were transferred to an aseptic 4 per cent yeast-agar medium, the females oviposited in one to three days. The larvae that emerged reached a length of 5 mm. in three days; the females remaining on the banana did not oviposit. Guyénot ('13, b) has explained this as a nutritional phenomenon. He observed that normal females from yeast-fed larvae placed upon a poor food, such as carrot, after a few days deposit eggs which though fertilized no longer develop to maturity, but die as partially developed embryos. If the same female recopulates after a period, it at first deposits normal fertile eggs, then abnormal fertilized eggs, and finally unfertilized eggs. The following experiments of Guyénot's ('13 d) will serve further to illustrate this point. He reared adults from aseptic larvae fed on sterile potato, but found that most of them were almost sexually sterile. Oviposition did not

begin till the females were seven to twelve days old (normal period thirty-six hours) and the number of eggs was 117instead of the normal 576 (24 per day). Only five larvae emerged, 49 embryos died owing to deficiency of the sperm, and 63 eggs were unfertilized. Anatomical examination by the author ('13 e) showed that only 20 to 40 eggs are normally formed in the body of the female at the time of emergence from the pupa. These are deposited in forty-eight hours, and after that all the stored material in the eggs, normally 24 per day, must be derived from the body and food of the insect. The effect of the food of the adult upon fecundity is very marked, thus 'non-fertile' sister adults from potato-fed larvae were placed, 1) on potato, where they laid one egg per day for 7 to 13 days and, 2) on potato and yeast, where they laid 10 to 15 eggs per day after 5 days and then 24 eggs per day. The converse experiment was to place sister adults raised from larvae fed on potato and yeast on 1) potato and yeast, where after 24 hours, 20 to 27 eggs per days were deposited for 10 to 17 days and, 2) on potato, where after 24 hours 20 to 27 eggs were deposited for 3 days and after that but 1 egg per day. These experiments all account for the death in the embryonic stage of eggs of a normal female, but the following experiment shows clearly that it is due to resorption by the female of the sperm cells in the bursa copulatrix (Guyénot, '13 b). 1) Adults from larvae reared on potato when placed on yeast laid from the 4th to 15th day 300 normal eggs, on potato after 7 to 13 days, 2 to 3 fertile eggs, later 20 eggs which died without hatching although fertilized, and finally 30 unfertilized eggs. 2) Adults from larvae raised on yeast, when placed on yeast deposited 24 eggs per day after 36 hours, and on potato, behaved the same as adult bred on potato, but the effect was slightly postponed.

The foregoing considerations show that the fertility of adults is a question of gross nutritional requirement and that it is difficult to interpret the yeast requirement in these cases, as a need of special substances. This is especially true since the accessory factors or vitamines which have been studied by Funk ('11), Osborne and Mendel ('13), Hopkins ('12), and others are necessary only in extremely minute quantities and are not used up in a

short period, as would have to be assumed in the case of the female Drosophila.

Guyénot ('17) has summed up all this work in a thesis and has added some experiments concerning the exact constituents necessary for a synthetic diet for Drosophila. In this he is successful to the extent that with one exception the components of an adequate diet are discovered. These are peptone, lecithin, inorganic salts, water, and an extract of yeast, the composition of which is unknown but appears to be a part of the yeast protein molecule. This extracted substance is most completely removed from yeast by boiling in 60 to 70 per cent alcohol and can be recognized by its solubility in boiling absolute alcohol, cold 70 per cent alcohol, and boiling and cold water. Attempts to substitute amino-acids, cleavage products of nucleoprotein, nuclein, carbohydrates, salts, organic acids, and fats for this special substance were all failures. Experiments with peptone gave best results when 4 per cent was used, but no larvae pupated unless lecithin was added, which permitted the storage of fats and pupation, but not the emergence of adults. The addition of bouillon to peptone also permitted a few abnormal pupae to be formed, but no adults emerged. Completely filtered autolyzed yeast, together with lecithin and peptone, made a complete and normal food for the insect. Liver autolyzed or extracted could be substituted for the yeast extract with equal success. The author also studied the formation of reserve fats and found that this process depended mainly on lecithin, but could go on to a slight extent at the expense of the protein derivatives in the yeast extract.

These results of Guyénot do not necessarily conflict with my own, as the special substance extracted by boiling alcohol is probably included in the nucleoprotein used in my experiment,[19] as Guyénot has pointed out. No fats were present in the yeast nucleoprotein used in my work, as I had extracted these with ether. As Guyénot found that lecithin is required, there would appear to be conflicting results in this regard, however, he also

[19] In drying, the nucleoprotein was washed with cold alcohol, but the special substance of Guyénot is not extracted unless the alcohol is boiling.

found that certain crystalline solids left on the filter after filtering autolyzed yeast could be substituted for lecithin. These crystalline substances are probably also constituents of yeast nucleoprotein.

Loeb and Northrop ('17) have recently used glucose beef agar for maintenance of larvae and adults so that the temperature coefficient of the duration of life could be determined, and Northrop ('17 a) has shown that the total duration of the life of Drosophila can be increased by retarding the growth of the larvae, as the pupal and imaginal periods do not seem to change with the increased larval life. These results are entirely comparable to those given on page 30. Northrop ('17 b) describes experiments which have led him to the conclusion that yeast supplies a special substance necessary for the growth of Drosophila larvae. This author finds that banana, casein, and sugar supplement yeast as a food for larvae and permit the development of a larger number of adults than could take place on yeast alone. The optimum mixture contained 33 per cent yeast, and as the amount of yeast decreased the number of adults reared became less and growth of larvae slower until at a proportion of yeast of 1:128 the growth of larvae became abnormal. Kidney, liver, and pancreas of dog were adequate foods for larvae, but spleen, heart muscle, muscle, blood, adrenal, and thyroid were not a complete diet for the insect. The author concludes that the special substance required for growth cannot be obtained from protein or carbohydrates. From my experiments I have evidence (p. 14) that banana and sugar have food value for Drosophila larvae, and to this extent my results are in accord with Northrop's, however, since the insects can develop normally on yeast nucleoprotein, sugars, and salts it seems probable that the special substances required for the growth of Drosophila are included in nucleoprotein.

In summing up the results of my experiment I conclude: 1. Drosophila normally feeds on fermenting fruit, obtaining a large part of its nourishment from the microörganisms, especially yeasts, which are in a loose symbiosis with the insect.

2. Dead or living yeast is a complete food for Drosophila.

3. The larvae are dependent on the nucleoprotein of yeast for special substances necessary in their growth.

4. The function of yeast in the ecology of the insect is to concentrate at the surface of the medium and to synthesize into nucleoprotein, the urates, ammonia, or amino-acids of the substratum.

2. Experiments with a sarcophagous insect

A pair of adult Acalyptrate muscid flies of the species Desmometopa m-nigrum Zett. (determination by Mr. C. W. Johnson) were received through the courtesy of Dr. W. M. Mann. They had emerged from some poorly dried snail shells, collected in the Fiji Islands, on the decaying flesh of which the larvae had fed. The adults were placed on banana and yeast agar, where the female deposited about forty eggs, most of which died owing to a thick mat of a black mucor that grew over the surface of the medium. The six larvae that emerged fed readily on the rich yeast food, and in about twenty-two days reached a size of 12 to 15 mm. in length. The black fungous mat was not destroyed and did not seem to injure the larvae. The six pupae formed were normal, and six adults emerged after three to five days and oviposited on the medium.

The usual manner of interpreting the normal feeding habits of this species would be to state that the larvae fed on decaying animal tissue. This, however, is open to doubt in view of the above experiments, and we must now consider the probability that all decaying or fermenting substrata are merely the media on which the fungus and bacterial food of the insect is growing.

3. Experiments with a coprophagous insect

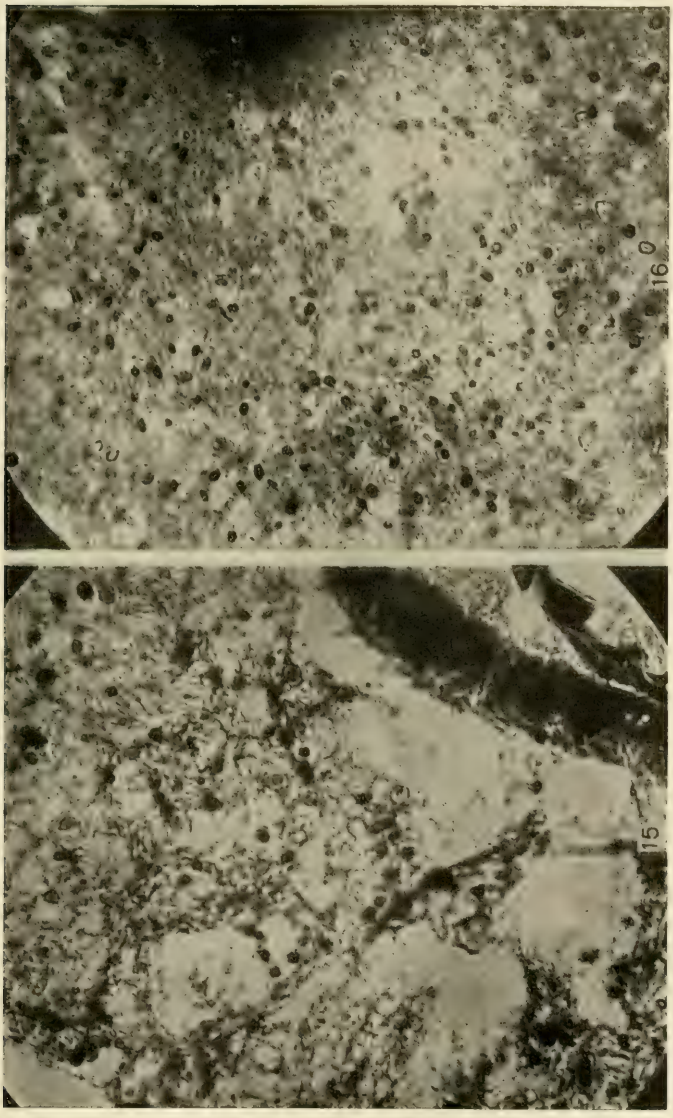
An investigation of the food of the housefly (Musca domestica) also gives support to this theory. The insects were obtained in winter by placing bran mash in the greenhouse. The mash was prepared by boiling "Educator" bran with an equal volume of water with constant stirring for twenty minutes. It was placed in a large porcelain dish in the hothouse, where it soon became

covered with Rhizopus nigricans and Penicillium glaucum molds. These growths were turned under each day and the bran thoroughly wetted till the mash had a sour smell and no longer became covered with the molds. Thus far no flies had deposited eggs on the mash, although large numbers of Lucilia caesar and Musca domestica fed on the moist surface. Several lumps of ammonium carbonate were then added, giving the medium an odor indistinguishable from manure. No eggs were observed, but larvae were soon found feeding just under the slightly incrusted surface. They gradually worked down in the medium as they became larger and the upper portions of the mash were dried out or the nutritional substances "burned" out by the fungus due to over-aëration. When this same medium was used a second time, larvae were seen to penetrate into hard lumps of the bran which still retained a visible white powdery appearance of fungous mycelia.

Two-day-old (5-mm.) larvae were transferred to bouillon yeast, banana and yeast, bran and Pasteur's agar media. The larval life was as follows:

Bouillon agar	5 mm. to pupation	13.5 days
Pasteur's agar	5 mm. to death	2.0 days
Bran agar	5 mm. to imago	8.0 days
Yeast agar	5 mm. to pupation	4.0 days
Banana and yeast agar	5 mm. to pupation	4.0 days
Bran mash	5 mm. to pupation	13–21.0 days

The larvae showed signs of great disturbance when placed in the Pasteur's medium where the sugars seemed to act as a poison to them. On bouillon agar the larvae were not very successful in completing growth and usually formed abnormal pupae; on the other agar media growth was more rapid than in the bran mash. This was due to the luxuriant growth of yeast, molds, and bacteria which the bran, banana and yeast agar supported and which served as food for the larvae. Sections through the larvae from the bran mash showed a complete absence of all material except bacteria, fungous spores, and yeast cells in the digestive tract. The microphotographs in figures 15 and 16 show the process of digestion of the microörganisms and leave no doubt that they



Figs. 15, 16 Serial cross-sections through the digestive tract of a Musca domestica larva showing the digestion of fungus spores (p. 44).

serve as food for the insect. Furthermore, the larvae are never found in any materials that are not infected with microörganisms and are in a process of decomposition or fermentation, and it is doubtful that the larvae find in these substrata nutritional substances which will be of great value to them, for the food materials are rapidly changed to decomposition products which are either absorbed by microörganisms or are too simple in composition to be available for the insect.[20] The real function of the microörganism is to synthesize protein from ammonia, urates, etc. Female flies seldom deposit eggs in substances which do not have the odor of ammonia, which as products of the action of yeasts, molds, and bacteria indicate the presence of the fungous foods of the larvae.

An attempt was made to sterilize Musca domestica pupae by washing in 85 per cent alcohol or in 85 per cent alcohol saturated with $HgCl_2$ for periods of from five to forty minutes, but in each of the 200 pupae used in the experiment, Aspergillus or Penicillium mold developed around the pupa. It would seem that the molds are carried within the pupae, although this is not definitely proved. In this connection the following quotation from Bogdanow ('08 b, p. 199) is of interest:

> Wenn Calliphoralarven bei Anwesenheit von Bakterien nur von Albumosenlösung ernährt werden können, so können die Larven von Musca domestica umgekehrt mit Stärkekleister oder mit Gelatine ohne Zusatz anderer Stoffe gefüttert werden, aber, soviel ich beobachtet habe, nur dann, wenn Schimmelpilze und Bakterien da sind.

Prowazek ('04) has found that Apiculatus fungi are usually present in the intestine of Musca and Sarcophaga larvae. A number of experiments have been performed to determine whether or not the pathogenic bacteria, with which housefly larvae become contaminated in their natural habitat, survive in the fly during the pupal period. Most of the results (Graham-Smith, '13) show that only such spore-forming bacteria as anthrax pass through the pupa alive. Tebbutt ('13) in this connection raised houseflies on agar, a little human blood, and living bacillus dysen-

[20] See page 48.

teriae (type 'Y') and B. typhosus. The fly eggs were sterilized by washing in 3 per cent lysol for two to three minutes. The pupal contents were plated and found usually to be sterile. Tebbutt does not mention the fact that the larvae probably obtained their nourishment from the bacteria.

From these experiments it appears very probable that the larvae of Musca domestica feed on microörganisms and are associated with them in the same manner as Drosophila and yeasts.

4. Experiments with a mycetophagous insect, Sciara, and a mite, Tyroglyphus, living in decaying wood

a. Experiments with Sciara. Through the courtesy of Mr. A. M. Wilcox who turned the material over to me, I was enabled to work on another fungus-eating insect found in twigs of the mountain ash apparently affected by 'black knot' or some dry black-rot disease. Under the bark and in the cambium of the wood slender white worm-like larvae, 12 to 15 mm. long, with a shining black head could be seen working in a glossy gelatinous sheath which they appeared to spin or secrete. As determined by Mr. C. W. Johnson, the larvae proved to belong to a species of Sciara, a genus of fungus gnats which feed in decaying vegetable matter and are pests on cultivated mushrooms.

The larvae were transferred to a medium of bran agar which they infected with a mucor, a Gleocladium, and a Fusarium. The larvae moved on the surface of the agar through the field of vertical sporophores with their black globular sporangia overhead. Occasionally one would raise its head and sieze a sporangium between its mandibles. The disintegrating sporangia could also be seen in the digestive tracts of the semitransparent larvae. The mandibles are peculiarly fitted for such feeding, as they are quadrate in form and having three large and several small interlocking teeth. The flat surface which the mandibles form would make it impossible to seize any structure not raised above the surface of the substratum. The larvae are also very fond of the mucilaginous secretions or exudations which appear as brilliant globules on the sporophores or sporangium walls and as a sheath around the larvae.

As mentioned above, the larvae appear to move in a gelatinous sheath over the wood. This habit has been observed in a number of fungus-eating Diptera and has been described by many authors as a secretion of the larvae. The following extract from Malloch ('17) will illustrate the present interpretation of the habit:

> Nearly all of the larvae (Mycetophilidae) spin webs in the galleries they make in their food; in the case of species that live externally upon fungi the web is slimy, rather loose and irregular. I have paid particular attention to some species I have reared, and find that the larvae of this group do not pass over the threads but through them as in a tube, the body enclosed except anteriorly. The threads are slimy in nature and the presence of the larvae may be detected by the glittering surface of the fungus which appears as if a slug had crawled over it (p. 250).

My larvae, feeding on mold in bran media, could be observed very closely under the binocular microscope. It was seen that in passing through the 'field' of fungus the larvae usually took a certain course, thus forming a 'runway' similar to that made by a rabbit in high grass. The sporangia of mucors are converted into a mucilaginous mass when the spores are discharged and the sporophores also secrete a sticky fluid, both of which stick to the surface of the larvae as they pass through the fungous growth. Thus a shining gelatinous sheath is formed through which the larvae pass. When moving over its course the larva 'flows' along in a large drop of liquid which completely surrounds the insect and assumes the same form. The surrounding drop, if stained with eosine and examined under the 1.6 mm. objective, proves to be a mass of spores of various kinds all arranged as though embedded in a clear unstained substance. A larva will often reach out and eat a portion of the surrounding drop of another larva that is passing. The sheath when stained shows a mass of mycelia growing from the spores embedded in the gelatinous matrix of fungous mucilage.

Brues ('02) has described the 'web' of Neoglaphyroptera opima Loew. and believes that it is spun by the larva which is found under the bark of fallen trees. As the insect is quickly killed by evaporation, he believes the web to be a protection against this danger. The larva was described as at times moving its head towards the web as though eating it.

From the foregoing observations on Sciarid larvae, it is apparent that they do not spin the web or secrete the gelatinous tube which surrounds them, but merely become covered with the exudations and spores of the fungi on which they live, and these spores, exudations, and hyphae serve as food for the insect. Upon pupation the enveloping drop of mucilaginous material surrounds the last larval skin and the pupa forming a cocoon of spores from which mycelia grow out. If the larvae are placed on a smooth paper under the binocular they are unable to move their long footless body. The mandibles with their flat surface cannot grasp the small particles of fiber which do not stand out above the surface. If a drop of water is placed upon the larva it immediately moves about actively by means of a ripple of circular contraction which starts at the posterior end and rolls a collar of integument over the anterior end. The anterior end is then protracted and the process repeated. It is apparent that in such a method of locomotion an enveloping fluid of high surface tension would be of great assistance. The function of the 'accidentally' accumulated mucilaginous envelope is twofold, first, to serve as a protection against evaporation and, second, to assist in locomotion.

The larval period is about twelve days and the pupal period four days. Adults are very active and run about rapidly, the male when in pursuit of the female flapping its wings vigorously. Adults may be seen to eject a hard white gelatinous body composed of fungus hyphae, etc. The adults and pupae seem much more immune to fungus attack than Drosophila. In the pupal stage this protection may be due to the complete envelope of gelatinous substance. The female deposits several separate piles of light yellow spherical eggs on the medium. These likewise seem to be immune to fungus injury as the mold often completely envelops them without causing death. The development and movement of the embryo can be observed through the egg which hatches in three to four days.

An attempt was made to sterilize the eggs and pupae, but death always resulted, probably owing to the soft exterior of these stages. The insects grew equally well on bran agar, yeast agar, and banana agar, feeding upon the luxuriant fungous growth always present.

The consideration of main interest in the present paper is the peculiar relation of substratum, microörganism, and insect which again finds an example in the food of this animal. It is is well known that molds contain enzymes capable of dissolving cellulose and hemicellulose, i.e., celluloses and cytases, which enable them to extend hyphae throughout the woody tissue of trees, etc., thus extracting all the nutritional substances. The nitrogenous natter is largely stored in the form of protein in the spores of the fungus, whereas the excess of carbohydrates may be excreted in the sticky drops of the sporophores. The insect feeding on the fungus, the wall of which it can dissolve, derives the benefit of the enzyme activities of the mold. If a section is made through larvae which have been feeding in the wood, it is seen that the great quantities of wood that pass through the digestive tract remain unchanged in structure. On closer examination fungous growths of an exobasidiomycete can be seen in the tissue cells (fig. 17). These fungi are dissolved out by the insect digestive enzymes and serve as food. The wood eaten by Sciarid larvae is therefore merely the substratum in which the fungous food material is embedded. This type of relationship is quite common among 'wood-eating' insects and is quite comparable to the symbiosis of Drosophila and yeast.

b. Experiments with a mycetophagous mite living in decaying wood. A mite of the genus Tyroglyphus (determination by Mr. N. Banks) was also found on decayed mountain-ash twigs and bred upon bran agar like the Dipterous larvae described above. Five mites added to the tube climbed about on the thick growth of fungus, apparently eating the spores in the muclaginous sporangia. As the mites rapidly multiplied the growth of the molds was checked and they were cleaned off the surface till only a few blisters or pustules of Fusaria remained. The mites could be seen to feed in large numbers at the edge of these pustules which served as food for two months allowing the mites to increase enormously in size and number. Many of this same genus of mites are known to feed on cheese, ham (on which powdery molds grow), and dry molds of various kinds, and manure, decaying fungi, and vegetable refuse are always inhabited by mites

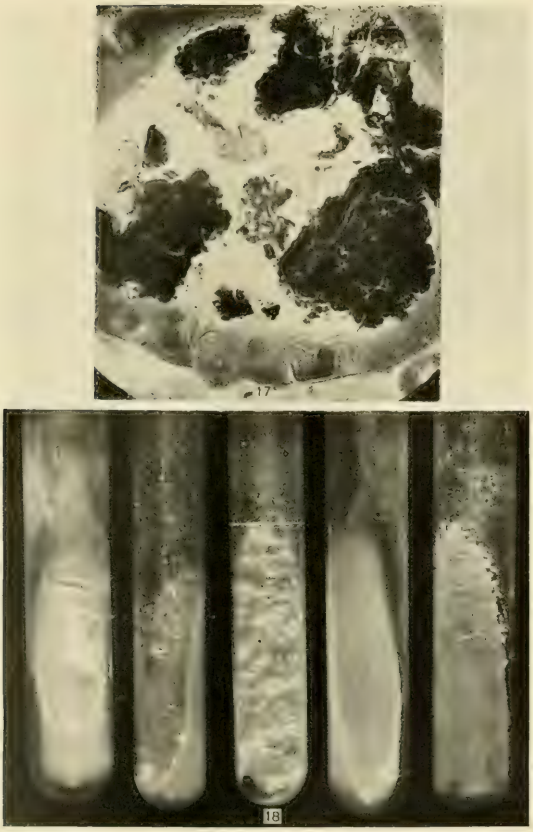


Fig. 17 Cross-section through the digestive tract of a Sciara larva showing the fungus mycelium on ingested woody tissue (p. 50).

Fig. 18 Agar Drosophila cultures (from left to right). 1st tube inoculated from second tube. 2nd tube, large larvae and pupae on non-sterile banana. 3d tube, small larvae of same age as 2 on sterile banana. 4th tube, inoculated from 3, showing its sterility. 5th tube, large larvae and pupae on 6 per cent dead yeast of same age as 2 and 3 (p. 11).

of various species. It is quite probable that mites inhabiting decaying and fermenting material, feed largely on the microörganisms present.

c. Association of wood-eating insects with fungi. The reason for the association of Sciara and Tyroglyphus with fungi is probably because of the indigestibility of the cellulose walls of the wood and the small amount of protein contained in them. The composition of wood varies with the season and with age, species, location, and tissue, so that it is difficult to make any general statement. Haberlandt ('15) has recently studied the digestibility of wood and concludes that unless the cellulose is destroyed or changed, wood has little food value for mammals, as the nutrient substances are inaccessible. Birch-wood was found to have the highest nutritive value, giving the following analysis: water, 4.56; nitrogen, 0.108 (protein, 0.675); ether extract, 0.45; nitrogen-free extract, 61.56; crude fiber, 32.2, and ash, 0.46. In general it may be said that, except in the living phloem and cambium, the protein content of wood is extremely low. Carbohydrates are usually abundant and wood is therefore used in the manufacture of sugar, some processes yielding as high as 25 per cent. These compounds are probably quite inaccessible to insects because of their chemical and physical nature, but are readily dissolved and converted by fungi due to their notable enzyme activities.

The low nutritive value of wood causes the insect to either lengthen its life-cycle so as to be able to extract a greater amount of wood or it leads to association with microörganisms either as food or as symbionts. In the first class belong the large majority of heartwood-boring Lepidoptera, Coleoptera, and Hymenoptera, for example, the moth larvae Zeuzera pyrina and Sesia apiformis with a two-year life-cycle; the larvae of the beetle Saperda populnea with a two-year, Elaterid larvae with a three-year, Melolontha vulgaris with a four-year life-cycle, and Sirex (Hymenoptera) with a larval period of one year. The next step which may lead to the habits of the ambrosia beetles and termites might be the reingestion of material already passed through the digestive tract as described by Escherich ('95) for the beetles of the family Ipidae (Bostrychidae) which he believes to be adopted

for the purpose of extracting all the nutriment possible from the food already comminuted. This would lead us to the case of the Cecidomyiid, Asphondylia prunorum, which was studied by Neger ('08 a, b; '10). The adult deposits with the egg on the prune tree a mass of fungous spores and mycelia which serve as food for the larva and finally grow on the tissue of the gall formed. The fungus itself is not concerned in the gall formation, but merely serves as food for the gall inhabitant. Upon the emergence of the adult insect the fungus breaks through the gall and can be seen as a white growth from the outside. The fungus, a Macrophoma species, is very similar to the fungus fed upon by the wood-boring beetles (Xyleborus, Xyloterus, etc.) and has never been found elsewhere; the galls have therefore been called 'ambrosia-gallen.'

At the pinnacle of this development may be placed the ambrosia beetles and termites. Schmiedberger ('36) first gave the name 'ambrosia' to a protein-containing white substance which he found to be the food of the insect rather than the chips of wood cut by them. This was made certain by the observations of Hartig ('44) and it was also decided that the white substance was a fungus which grew on the wood cuttings. The subject was further studied by Hubbard ('97) in America and Neger ('07) in Germany. Though these two investigators do not agree in all their observations, they have made certain that different species of fungi are associated with different species of beetles and that these associations are constant for the same species in spite of changes of host plants or parts of plants eaten (Xyleborus saxisenii). The fungus is independent of the food plant, but dependent on the products of the insect. Hubbard maintains that the female consciously carries the spores of the fungus to the new gallery and sows them. Neger, however, believes that the spores become attached to the highly sculptured wing cases of the female as it leaves the larval gallery, the walls of which are coated with the fungus. The fungus that grows on the walls of the galleries is different for different beetles, but in general is composed of either a chain of round cells which are assembled in an irregular heap or of upright threads with a round corpuscular cell on the

tip. The latter condition is termed conidial by Hubbard. Escherich observed that these conidia served as food for the beetles and their larvae. As a certain degree of moisture is required by the fungus, the insects never select dried-out trees for their galleries, but always bore in wood which retains some sap. Other species of ambrosia beetles (Corthylus) are able to live in sapwood in the absence of the fungus, perhaps because of the abundance of protein in that region. The fungus is usually propagated in a little bed of chips, prepared by the female, in which the egg is deposited. In other species the woody tissue, after passing through the digestive tract of the larvae, has a yellow mustard color. In this condition it is plastered on the walls of the gallery and serves as a medium on which fungus grows. Undoubtedly, under these conditions the fungus may be considered as a chemical collaborator digesting food indigestible to the insect and furnishing it in a tender luscious form to the larvae.

Hedgcock ('06) studied fungi from various species of Ambrosia beetles and was able to refer them to the wood-bluing (Ceratostomella), wood-blackening and -browning (Grophium, Hormodendron, and Hormiscium) and to the wood-reddening (Penicillium and Fusarium fungi. According to Escherich and Neger, the fungus in the absence of the larvae may assume a slightly different form. Neger has also found fungi in the galleries of Cerambyx and Tetropium luridum, but is unable to decide whether or not it serves as food.

The fungus-growing habits of the white ants or termites are principally known through the work of Hagen ('60), Holte ('99), Haviland ('02), Trägardt ('04), Doflein ('05, '06), Petch ('06), and many others. These wood-eating insects build subterranean nests, the ground which they excavate being placed in a pile which itself is later used to form chambers. Shafts may be left in this superstructure with an outer chimney; these are used as permanent scaffolds and have little effect on the ventilation of the nest. The fungus gardens are either on the floor or suspended from the ceilings of the chambers, and consist of a mass of comminuted woody tissue which has passed through the digestive tracts of the workers and is then built into the comb. The fun-

gus which grows on this comb (as in the case of ambrosia beetles, really upon food cut by the insect, but largely indigestible to them, is described by Petch ('06) as follows:

> The mycelium on the comb bears small, white, stalked or almost sessile 'spheres.' These consist of branching hyphae bearing either spherical or oval cells. The spherical cells do not germinate. The oval cells germinate readily but it has not been possible to reproduce the 'spheres' from them. When the comb is old an agaric grows from it. This agaric appears in two forms, one of which has been assigned by various mycologists to Lentinus, Collybia, Pluteus, Pholiota, and Flammula, and the other to Armillaria. It develops in a cartilaginous, almost gelatinous, universal veil and is a modified Volvaria. Other fungi which grow on combs removed from the nest include Mucor, Thamidium, Cephalosporium, and Peziza. As these are not found in the nest, though some of them are capable of development underground, it is probable that the termites 'weed out' foreign fungi from the cultivation of the comb. *The comb material is probably sterilized by its passage through the alimentary canal.*[21] That the spheres form the food of the termites is probable, as in the case of the leaf-cutting ants; neither case can be considered definitely proved. *Termes redemanni* and *T. obscuriceps* undoubtedly *prefer fungi, or wood which has been attacked by fungi* . . . . It is most probable that the 'spheres' in the termite comb and the 'Kohlrabihäufchen' of the leaf-cutting ants investigated by Möller are parts of a normal mycelium, and that their shape is modified by the insects only in a very slight degree, if at all . . . . The available evidence appears to show that the 'spheres' are part of the mycelium of the Volvaria, but it has not been possible to connect these forms experimentally.

The fungus gardens are found in all chambers except the royal chamber; here the queen lies in state and is fed (Doflein) with a concentrated and easily assimilated food consisting of mycelial spherules by the workers. The larvae, according to Petch, do not show the presence of any spherules in the digestive tract, but may be fed on some regurgitated or predigested food furnished by the workers, which in turn feed on decaying wood. It is of interest that the queen termite is the only known adult insect which increases in size. The queen is usually the center of a pool of fatty secretions on which the workers feed with great satisfaction.

[21] My italics.

Undoubtedly these complicated habits have come about by taking advantage of the enzymatic and possibly synthetic power of the microörganisms. The type of association is the same as with Drosophila, but is further complicated by the physical properties of the wood and the habits of the insect.

Some insects that feed on wood are apparently not associated with any microörganisms, for the burrow does not appear to be discolored. However, as a general thing, larvae are in symbiosis with some microörganism when boring in woody tissue. When feeding on leaves larvae are often completely or reasonably sterile, and this is what would be expected from the foregoing assumptions, for the tissue of the leaves is very soft and readily digested.

Portier ('05) has thrown some light on this subject by his studies on the caterpillar of Nepticula, a small lepidopterous insect that feeds in the parenchyma tissue of rose leaves. The eggs are deposited on the leaf, which he supposes is sterilized by the sun's rays, and the larva bores directly down into the leaf, sealing up the entrance; the feces are not thrown out as in the case of Tischeria. The exterior of the leaves were sterilized by Portier and the whole leaf, with the excavation cut open, was covered with bacterial media. The fifteen cases investigated were perfectly sterile[22] whereas all cases of species which throw out the feces (Lithocolletis and Tischeria) were contaminated with bacteria and fungi, especially Aspergillus niger. Later ('11 a) Nonagria typhae larvae, that live in the trunk of Typha latifolia, were investigated, and it was found that they were associated with a pseudobacillus present in all tissues of the body, having passed through the chitinous peritrophic membrane during ecdysis. The bacteria were in all stages of decomposition in the phagocytic cells of the blood. A second paper ('11 b) showed that in Nonagria typhae a more complex situation exists than at first described, in which two microörganisms are in symbiosis: a micrococcus and a fungus Mucidium (Isaria). This fungus must

[22] During the summer of 1917 I examined the digestive tracts of some thousand Porthetria dispar caterpillars, pupae and adults, and found microorganisms present only in pathological cases, therefore this insect is not associated with fungi.

be held in check by the larvae, since if allowed to sporulate, as occurs after death, injury would result. Portier believes the secretion of the labial glands have this function. To link these observations with the case of Nepticula, in which the larvae are aseptic throughout life, Portier ('11 c) describes the condition in Gracillus syringella, which at first feeds in an aseptic condition on the soft interior of the leaf, and then, after feeding on the exterior of the leaf and becoming associated with a digestive flora[23] capable of dissolving woody tissues, bores into the twigs, the organisms being in part absorbed by phagocytosis as food for the larvae.

Internal symbionts have also been found in a beetle, Anobium paniceum, by Karawaiew ('99) and Escherich ('00). These symbionts always occur in definite cells in the anterior end of the midgut. Karawaiew thought he recognized a vacuole in them and therefore considered them to be Flagellates. Escherich, however, studied them in hanging drops of sugar solution and determined that they were Saccharomycetes. As these yeasts always occur in the same cells and pass through the pupa into the adult, it is quite likely that they are transmitted through the egg from one generation to another. Escherich found that the number of yeast cells varied with the amount of nourishment taken in the different stages of the insects' metamorphosis, thus they were very numerous in the larva, rare in the pupa, and few in the adult. He therefore concluded that the fungi are intimately concerned with the nutrition of the insect. As the Anobiid feeds mainly on very dry house timbers, the symbiosis with a fungus could very well be of value to the insect in the extraction of food from the wood.

In general we may conclude that insects overcome the disadvantages of the chemical and mechanical composition of wood by association with microörganisms either as food or as internal symbionts.

[23] Henseval, M. (compare Biedermann) ascribes an antiseptic property to an essential oil secreted by Cossus ligniperda larvae. This oil has the property of making the wood more workable ('angreifbar').

EXTENT OF MYCETOPHAGY AMONG INSECTS

As a corollary to the foregoing conclusions we may assume that the foods of many insect larvae feeding on dead, decaying, and fermenting vegetable and animal matter are the micoörganisms which live upon the substratum in which the insects are embedded.

The extent of this habit among insects is very great, including a large number of Coleoptera and an especially large number of Diptera. This habit is usually apparent from the habitats selected by the insect, thus Metcalf ('16) lists the following habitats for the scavenger short- and long-tailed filth larvae of the flower-fly (Syrphidae), viz.: In decaying parts of trees and herbaceous plants, diseased or flowing sap, heaps of turf or soft mud containing vegetable matter, and in stagnant or putrid water, sewage, manure, or human feces. The larvae also occur as accidental body parasites, causing intestinal, nasal, auricular, and vaginal myasis. Some species serve as scavengers in the nests of termites, ants, wasps, and bees. It is apparent that microörganisms abound in all these environments, with the possible exception of the animal body In the latter case, however, it is well known that a foul odor, indicating some bacterial action, always precedes infestation. In more normal habitats the microörganisms so completely outweigh the other nutritive materials that it is quite likely they (the bacteria) serve as food.[24]

Townsend ('93) lists the following habitats for some of the scavenger Acalyptrate muscid larvae: dung, decaying wood, under bark, plants, leaves, roots, tubers, and fungi; in salt or alkaline water and mud; urine, vinegar, sap of wounded trees; cheese and animal fats. Again in this case all habitats selected by the fly normally abound in microörganisms, and it is quite safe to assume that they (the fungi) serve as food for the insect larvae.

The great extent of the use of microörganisms as food among insects is shown in a table of the feeding habits of larval and adult Diptera. In this table I have assumed that the food of insects, that always inhabit substrata of a fermenting or decaying nature,

[24] Osborne and Mendel ('14) showed that the bacterial content of feces was 20 to 40 per cent.

GROUP	LARVAL FOOD				ADULT FOOD				
	Saprophytic fungi	Algae	Higher plants	Animals	Saprophytic fungi	Fermentation products	Higher plant juices	Nectar	Fluids of animals
Tipulidae	x	x	x		?	?		x	
Ctenophorinae	x						x	x	
Tipulinae	x							x	
Limnobiidae								x	
Cylindrotominae		x	x						
Limnobiinae	x	x			x			x	
Limnobia	x							x	
Pediciinae		x		x				x	
Limnophilinae	x	x							
Limnophila	x	x							
Eriopterinae									
Helobia	?							x	
Gnophomyia	?							x	
Hexatominae	x	x							
Trichocerinae									
Trichocera	x								
Ptychopteridae	x	x						x	
Rhyphidae	x				?	?		x	
Boletophilidae	x								
Mycetophilidae	x				?		x	x	
Leia	x								
Exechia	x							x	
Sciaridae	x							x	
Platyuridae	x							x	
Psychodidae	x	x			x	x		x	x
Blepharoceridae		x						x	x
Culididae	x			x			x	x	x
Dixidae		x						x	
Ceratopogonidae	x								x
Chironomidae	x			x				x	
Orphnephilidae	x			x					
Bibionidae	x							x	
Scatopsidae	x				x	x		x	
Simuliidae		x							x
Stratiomyiidae	x	x		x				x	
Stratiomia	x	x		x				x	
Odontomyia	x	x		x				x	
Oxycera	x	x		x					
Geosargus	x								
Microchrysa	x								
Eupachygaster			x	x					
Xylophagidae			x	x			x	x	
Coenomyiidae	x			x			x	x	
Tabanidae	x			x					x
Coniops	x			?					x

GROUP	LARVAL FOOD				ADULT FOOD				
	Saprophytic fungi	Algae	Higher plants	Animals	Saprophytic fungi	Fermentation products	Higher plant juices	Nectar	Fluids of animals
Tabanus	x			x					x
Leptidae	x			x				x	
Atherix								x	
Chrysopila	x							x	
Cyrtidae				x					
Asiloidea				x				x	x
Mydaidae				x				x	x
Asilidae				x				x	x
Dasyllis				x				x	x
Bombyliidae				x				x	
Therevidae				x				x	
Scenopinidae	?			x				x	
Empidiae	x			x				x	x
Drapetis	x								x
Dolichopodidae				x				x	x
Phoridae	x			x					
Platypezidae	x								
Bipunculidae				x					
Syrphidae	x			x				x	
Conopidae				x				x	
Psilidae			x						
Sepsidae	x				?				
Trypetidae	x		x						
Sapromyzidae	x								
Agromyzidae			x						
Geomyzidae			x						
Drosophilidae	x				x	x			
Ephydridae		x	x						
Oscinidae			x						
Phycodromidae	x								
Borboridae	x	x			x	x			
Heteromeusidae	x								
Helomyzodae	x				x				
Cordyluridae	x		x	x					
Anthomyidae	x		x	x					
Muscidae	x			x	x	x		x	x
Oestridae				x					x
Sarcophagidae	x			x	x	x			
Dexiidae				x					
Tachinidae				x					
Hippoboscidae				x					x
Streblidae				x					x
Nycteribiidae				x					x
Sciomyzidae	x	x							

is the microörganisms themselves, and to a less extent the substratum. This assumption can safely be made on the basis of the preceding experiments and the general lack of nutritive value (for insects) of many of the substrata concerned. This interpretation of the food of scavengers, etc., has never been given before to the author's knowledge except in the case of Drosophila, as mentioned above (Guyénot, Loeb, Schulze, Henneberg). The data in the table are mainly derived from Malloch ('17) and Williston ('08). From a glance at the table it is apparent that a large majority of the Diptera live upon microörganisms. Therefore it might be well to include under the term Mycetophages all insects which have hitherto been termed scavengers, coprophages, etc.

Parrott, Fulton, and Gloyer ('14, '15, '16) found that tree crickets eat fungous "mycelia and spores which are unaffected by the intestinal juices."[25] This assumption is based on the fact that the spores of fungi are not digested by Oecanthus, as germination takes place from pellets of material passed through the digestive tract, and they believe

> it is possible that the spores may act as roughage and were eaten for that purpose; but it appears more plausible that the spores still retain on their surface some of the protoplasm of the ascus or pycnidium which makes them palatable ('16, p. 12).

The assumption that fungi are not digested, based as it is on the evidence that many living spores pass through the digestive tract, is not entirely justified in my opinion, since the same can be said of yeast cells in the Drosophila larvae, although in this case the plant undoubtedly serves as food.

The leaf-cutting ant is a good example of a mycetophagous insect. The fungus-growing habits of these Attiine ants should really be depicted at the same time as the habits of termites, as

[25] The wound made in tree twigs in which the eggs are placed is plugged with excrement and often becomes the seat of the development of cankers. Gloyer and Fulton ('16) give a concise account of the literature on the dissemination of plant diseases by insects. This is in large part due to the habit of insects of feeding on all available fungus and bacterial growths and in doing so getting the body covered with spores which readily remain attached to the hairs and spines on the surface of the body.

they are equally specialized. However, the origin of the habit appears to be different, as will be seen later, therefore their fungus gardening is best described at this point. Our knowledge of these forms is specially due to the work of Bates ('63), Belt ('74), Tanner ('92), and others. These ants excavate subterranean nests composed of a series of chambers connected by a vertical shaft usually ending in a crater. The fungus garden is built of the comminuted fragments of leaves, cut from trees near the nest, which have not passed through the digestive tract. The little pellets thus formed are built up into the sponge-like mass suspended from the ceiling or placed on the floors of the chambers. Caterpillar droppings are quite commonly built into the comb, which serves as a substratum for special kinds of fungi. A summary of all the literature on these ants, the termites and the ambrosia beetles, as well as some important contributions to the ethology of American species, is given by Wheeler ('07), from which I quote the following conclusions of Möller:

> All the fungus-gardens of the Atta species I have investigated, are pervaded with the same kind of mycelium, which produces the 'kohlrabi clusters' as long as the ants are cultivating the gardens. Under the influence of the ants neither free aerial hyphae nor any form of fruit are ever developed. The mycelium proliferates through the garden to the complete exclusion of any alien fungus, and the fungus garden of a nest represents in its entirety a pure culture of a single fungus. The fungus has two different forms of conidia which arise in the garden when it is removed from the influence of the ants. The hyphae have a very pronounced tendency to produce swellings or diverticula, which show several more or less peculiar and clearly differentiated variations. One of these, which has presumably reached its present form through the influence of cultivation and selection on the part of the ants, is represented by the 'kohlrabi heads.' Under artificial conditions the 'kohlrabi clusters' and 'heads' disappear and the fungus becomes a mass of bead-like conidia.

Sampaio ('94), von Ihering ('98), Goeldi ('05), and Huber ('05) have shown how the new fungus colony is started after the marriage flight. The queen deälates itself, digs a small subterranean burrow which it closes and then starts the new colony by spitting out a pellet of fungous hyphae which had been carried in the buccal pocket and depositing eggs upon it. The fungus

colony is grown upon the liquid excrement of the female, which touches small pieces of the mycelium to the tip of its gaster and then replaces them in the fungus garden. Some of the eggs are sacrificed as manure for the fungi.

Parrott and Gloyer ('16) are of the opinion that the fungus-growing habit of the Attii may have come about through the collection of caterpillar feces in which many spores accidentally eaten by the larvae are still alive. They do not give any explanation of the habit of collecting the feces, however. Wheeler ('07) points out that the gardens are usually composed of a substratum consisting largely of fecal material in the case of the ambrosia beetles and termites, and that the habit is pronounced in the lower genera of leaf-cutting ants and visible in all cases closely studied. It therefore seems probable that the food of the Attii "may have been originally grown on fecal substances" Von Ihering ('94) believes that the habit may have phylogenetically originated with ants using moldy seeds stored as food. I should suggest the possibility that the ants may originally have fed upon fungous mycelia developing on caterpillar feces from spores unkilled in their passage through the digestive tract. Such droppings might finally be carried into the nest where the fecal substratum and the moisture of the nest would soon allow the growth of a valuable crop of fungous food. Thus the fungus-growing ants developed their habits as a direct response to a valuable food supply. On the other hand, the termites developed their habits as a means of making use of an unmanageable food supply.

The examples cited above indicate that the use of microörganisms as food[26] is widespread among insects and is a direct response to the high food value of the fungous cells. The feeding habits may be grouped into three classes as follows:

1. Ingestion of microöganisms with substratum, i.e., Drosophila, Musca, Sciara, worker termites.

2. Feeding directly on microörganisms, i.e., mites, tree crickets, many adult Diptera, etc.

[26] See page 68 for case of mosquito larvae feeding on fungi.

3. Preparation of medium for microörganisms, i.e., leaf-cutting ants, termites, ambrosia beetles.

MICROÖRGANISMS AS LIQUEFIERS OF THE SUBSTRATUM

The relation of the insect, microörganism, and substratum is not always as clearly defined as in the preceding cases. Fabre ('94) studied the food of Lucilia, the green-bottle fly, and came to the conclusion that the larvae secrete a digestive fluid which allows the liquefied albuminous material to be sucked up by the insect. He placed in one tube of hard-boiled eggs a few fly eggs and left the other tube of albumin equally exposed to the air, as a check. The albumin on which the larvae emerged was soon a liquescent mass, whereas the check dried up. Guyénot ('07) reinvestigated the problem with Phormia regina Meigen and also studied the anatomy of the larvae. The mouthparts as he describes them are very similar to those of Drosophila larvae, as mentioned above. The pharynx is immediately connected with the crop of sucking stomach, a much distended flask-like structure which usually lies to one side of the oesophagus. Owing to the nature of the pseudomaxillary apparatus, the larvae are unable to eat any solid food. Fabre had supposed they secreted on the food some pepsin which liquefied the albumin. To test this theory, Guyénot ground up the larvae and made various extracts. The extracts had no effect on starch, fat, or albumin; the same was true of extracts of the salivary or gastric glands. The normal liquefaction was then studied, and it was found that the albumin was broken down to the peptone stage by a bacterium Micrococcus flavus liquefaciens (Flugge) which was always present with the larvae. The bacteria alone, without the acid of the larvae had the same effect on the albumin, but at a much slower rate. However, if they were mixed with the albumin with a sterile platinum wire the speed was as great as with the larvae present. As these bacteria were found in large quantities in the sucking stomach of the larvae, Guyénot reached the following conclusions:

I. La liquéfaction des substances albuminoides résulte d'une véritable digestion opérée par certains microbes de la putréfaction.

II. Les larves de mouches, absorbant exclusivement des aliments liquides, directment assimilables, ont un travail digestif réduit au minimum et ne produisent pas de ferments solubles en quantité appréciable.

III. Les larves accelérent la putréfaction des cadavres en favorisant la pullulation des microbes.

IV. Les larves se nourrissent aux dépens des produits du chimisme microbien; les microbes ne peuvent se développer rapidement que s'ils sont repartis en tous points par les larves. Il existe entre ces deux agents de la putréfaction une véritable symbiose (p. 369).

Guyénot does not consider that the food of the larvae may be the microörganisms themselves, and this question is still open, however, unlike the following example, the microörganisms associated with Lucilia have the function of liquefying the food material.

Bogdanow ('06) studied the similar case of Calliphora vomitoria, the flesh fly. The eggs were sterilized by washing for two one-and-one-half-minute periods in 5 : 1000 aqueous $HgCl_2$ solution and then rinsed in running sterile water and were then placed on sterile media of casein, egg albumin, etc. None of the flies obtained was sterile, but was usually associated with a micrococci which Bogdanow believed was passed through the egg. The larvae grew rapidly on casein, egg albumin, albuminoides, etc., in the presence of micrococci and a gelatin-dissolving bacterium, but the flies that emerged were few in number and very small in size, being 'starvation forms.' The larvae were later given the selection of fresh or putrid meat, showed a preference for the former, the putrid meat usually killing the larvae. Meats putrifying in the presence and absence of larvae could be distinguished by a difference in odor as the micrococcus with which the insect infects the meat liberates ammonia from proteins. The larvae grew normally on meat in the presence of a gelatin-liquefying bacterium and the micrococcus. Two factors are therefore necessary for the successful metamorphosis of the larvae the micrococcus from the egg and a gelatin liquefier from the air.

In 1908 (b) Bogdanow published a second paper on the same subject in which he showed that about 35 per cent of the eggs of the flesh fly are infested with a pure culture of micrococcus. The

other 65 per cent are sterile and can be reared on nutrient gelatin in the presence of a bacterium capable of its liquefaction. These larvae seldom result in normal-sized adults and it was not possible to raise sterile larvae on a synthetic medium of meat ash, peptone, and meat extract, acid or alkaline. Therefore Bogdanow concluded:

1. Im einfach sterilisierten Fleische wächst die Calliphoralarve gewöhnlich sehr schlecht.
2. In sterilisierten Resten der Larvennahrung wächst sie nicht besser.
3. Für die gute Larvenentwicklung sind meistens gelatineverflüssigende Bakterien oder Trypsin nötig (p. 193).

As nutrient gelatin is a highly inadequate diet for Drosophila, it is probable that the Calliphora reared on this medium obtained some of their food requirements by digesting the cells of the gelatin-liquefying bacteria which are unavoidably ingested by the larvae. As the flies were all undersized, it appears that this microörganism is at least not a complete food for the insect (as yeast is in the case of Drosophila). Therefore, Bogdanow's conclusion, that bacteria play merely the part of liquefiers in the ecology of the larvae, is largely warranted.

Wollman ('11) repeated Bogdanow's experiments and came to the conclusion that sterile Calliphora vomitora larvae can be rereared on sterile meat which has been sterilized by Tyndalizing rather than autoclaving. Bogdanow had autoclaved the meat used in his experiments, thus coagulating the proteins and making them insoluble to the larvae which when small have (according to Wollman) low proteolytic power. Though Wollman found that the larvae grew more successfully in the absence of microorganisms, as putrefactive bacteria always occur in the habitat of the fly, it is quite likely that they have some food value for the insect. Nevertheless, Calliphora, unlike Drosophila or Lucilia, grows best in the absence of all microörganisms.

ODORS ATTRACTIVE TO INSECTS

The odors which are attractive to dipterous adults are usually fermentation or decomposition products of the activity of micro-

organisms on a substratum. If we assume that the insect larvae feed upon these microörganisms, the chain of circumstantial evidence is complete. Our knowledge of the odorous substances attractive to flies has been advanced greatly by the work of Barrows ('07) and Richardson ('16a, b; '17), but these authors have never given the above interpretation to the response. Barrows studied the odors to which the adults Drosophila ampelophila (melanogaster) responds and found that the most attractive odors are those of ethyl and amyl alcohol, acetic acid, lactic acid, and acetic ether. A small amount of acetic ether, isobutyl acetate, methyl acetate, acetic or butyric acid added to ethyl alcohol greatly increased its attractiveness. "Alcohol and acetic acid are commonly found in cider vinegar, fermented cider and California sherry in per cents that are close to those which call forth the largest number of reactions in Drosophila." This odor is identical to that produced by the wine yeast Saccharomycetes ellipsoideus which I have found to cause females to deposit the largest number of eggs.

In 1916 (a) Richardson reported that he had carried on a series of experiments with odorous substances as baits for houseflies. The baits tested were placed under wire-gauze traps and were as following: Ammonium carbonate, ammonium sulphide solution, ammonium hydroxide, ethyl alcohol solution of skatol and indol, ethyl alcohol, acetic, formic, butyric and valerianic acids, hydrogen sulphide solution and carbon dioxide. "Negative results were obtained in all but the ammonium hydroxide and ammonium carbonate experiments." The ammonia was the attractive substance especially to females, which were found in the percentage of 89.2 to 7.5 of the males, although the actual percentage of sexes in the vicinity was 54 to 45.9 respectively. Valerianic and butyric acid augmented oviposition; the females, however, showed some discrimination between nutritious and non-nutritious material. In 1916 (b) a second paper gave a list of insects attracted to the ammonia, all of which spend at least part of their life in some form of animal excrement.[27]

[27] A third paper ('17) showed that aqueous solutions of carbohydrates are far less attractive than alcoholic or acetic acid solutions of such substances.

As both Drosophila and Musca domestica feed on microörganisms, it is of peculiar interest that the odors which stimulate oviposition by the female are identical to those formed by microorganisms in the substratum in which the insect normally breeds. The response of the female fruit fly to the odors of alcohol and acetic acid would indicate an instinctive response to the conditions best adapted to larval life, i.e., active fermentation. In the same way the response of the female housefly would indicate that the best conditions for housefly larvae require the presence of proteolytic (hence odors of ammonia, etc.) and fermentative (hence odor of alcohol and acetic acid) microörganisms. Richardson's results therefore give circumstantial support to my conclusion that the larvae of Musca domestica live on microörganisms.

Response to the odor of microörganisms is highly developed in the larvae of the yellow-fever mosquito, Stegomyia fasciata, as Bacot ('17) has recently shown that the eggs of this insect will remain for several months unhatched with the fully developed larva inside if the bacterial content of the surrounding water is low. The addition of foul contaminated fluid causes hatching in ten minutes. It is true that a fall of 6 to 10°F. causes some larvae to emerge, but the percentage is very low. Eggs were sterilized and transferred to sterile fluid, but if living yeast or bacteria were added they hatched immediately. Sterile autolized extract of brewer's yeast had the same effect, but killed bacterial cultures or watery extracts of yeast were ineffective. Bacillus coli was always effective when alive. The acidity and alkalinity of the different solutions were controlled. The author attributed the phenomena to the sense of smell of the larva and gave an exhibition of larvae feeding on stained bacteria (p. 178).

A rapid succession of different fungi occurs on manure (Gloyer and Fulton, '16, p. 6) and on other decaying substances together with an accompanying variety of odorous by-products. This succession determines the regular order in which decaying animal bodies become infested with insect scavengers. Mégnin ('85) and Hough ('97) have found that the order in which insects attack a decaying body is so constant that they have been able to develop a table giving the sequence of the different species. Thus

there are three stages of putrefaction and a final stage in which the dried tissues are consumed. The first stage of putrefaction is divided into two parts, viz., 'Body still fresh;' fauna consists of Musca domestica, Calliphora, etc.; 'Putrid odor develops;' fauna consists of Calliphora, Lucilia, Sarcophaga, etc. The workers of the second stage of putrefaction when butyric fermentation is taking place are Dermestes, Necrobia, Anthomyia, etc. The third stage, the stage of ammoniacal fermentation, is accompanied by infestation with such forms as Silpha, Necrophorus, Hister, Aphyra, Phora, and many Acarina. Finally the dried tissues are consumed by Aglossa, Tinea, Anthrenus, etc., and the bodies of these are destroyed Ptinus.

As this succession of species in the fauna of dead bodies holds fairly constant, it seems plausible that the odors produced are the determining factor and that the microörganisms producing the odors are of great importance to the insect as food and as solvent agents. Therefore we may conclude that the odors of fermentation and putrefaction are attractive to insects because they indicate a substratum made suitable for the insect by the abundance or the action of microörganisms.

MICROÖRGANISMS AS FOOD OF OTHER ANIMALS

The use of microphytes as food is not confined to insects and mites alone, but is quite common among Protozoa. The effect of pure culture of different species of bacteria on Paramecium has recently been described by Hargitt and Walter ('16). These authors were able to sterilize the animal by six successive washings in sterile water and then raised them on pure cultures of thirty different species of bacteria. They found that the bacteria from fresh were more favorable than those from older infusions.

It is probable that many of the Nematoda are also mycetophags. Anguillula aceti, the vinegar eel, which inhabits the 'mother of vinegar' and is also found in sour flour paste, and many of the 'parasitic' nematodes found in decaying plant tissue may be attracted at first by exposed soft tissue and later feed

upon the microörganisms in the decay with which they infest the plant.

Since Darwin's work ('81) it has been assumed that the earthworm finds its food in the humus of the soil it infests. Humus soil is notably rich in microörganisms, for these are the elaborators of humus from plant and animal remains, and it is possible that they are of food value to the worm.

Such structures as the endostyle and dorsal pharyngeal groove of Amphioxus and the Tunicates are probably for the purpose of entrapping microphytes of various kinds.

As pointed out by Osborne and Mendel ('14 b), microörganisms may also be of value to higher animals as elaborators of protein in the digestive tract from the non-protein substances ingested. This would be especially true in herbivorous animals, as Armsby ('11) has shown that non-protein substances are a source of protein in these animals, probably due to the formation of digestible bacterial protein in the digestive tract. The possibility that the flora of the digestive tract may modify the food elements supplied in a nutritional experiment is a drawback to the use of mammals in such experiments. An insect like Drosophila should be of value as material for such experiments because of the ease with which it is sterilized.

Many attempts have been made to rear mammals under sterile conditions, but most of these have failed so that it has been a great question whether or not it is possible for animals to live in the complete absence of microörganisms. As a large flora normally occurs in the digestive tract, it was necessary to sterilize the animal before it had taken food and to keep it in a sterile environment, therefore Pasteur ('85) suggested the use of hens, the eggs being well fitted for sterilization. Pasteur's suggestion was later carried out, but the first experiments were made by Nuttall and Thierfelder ('95–'96) on guinea-pigs, the young being removed aseptically from the mother by cesarian section. The animals were kept in a complicated aseptic environment and were fed upon food of animal origin. The animals gained 10 grams in one week (84 grams total weight) and appeared to be normal on the eighth day, when the experiment had to be discontinued.

In 1896 to 1897 the experiment was repeated, the gain in weight in eleven to thirteen days was almost normal, being 108 to 132 instead of 130 to 180 grams. In a third paper ('97) the authors experimented with the hen's egg and found that it was not sterile; they also summed up their previous work in the conclusion that animals just born do not grow well in the absence of microorganisms. Schottelius interprets the increase in the weight due of guinea-pigs in these experiments as due to the coagulation of caseinous material, from the milk, on the lining of the digestive tract. This author used hen's eggs as material to sterilize, but after a brilliant series of experiments ('99, '02, '08) has arrived at the conclusion that normal life without bacteria is impossible, as all the sterile individuals reared are retarded and stunted. Mme. Metchnikoff ('01) and Moro ('05) obtained similar results with tadpoles. In 1908 Tibbert, from theoretical considerations, came to the conclusion that higher organisms cannot live in the absence of microörganisms because each species of animal harbors definite numbers and species of bacteria. Metchnikoff, Weinberg, Pozerski, Distaso, and Berthelot ('09), on the other hand, reared the fruit bat Pteropus medius to normal size under practically aseptic conditions, and Cohendy ('12) kept chicks alive in anaseptic condition from twelve to forty days. Cohendy's conclusions are as follows:

> La vie sans microbe est possible pour un vertébré—le poulet—pourvu normalement d'une riche flore microbienne.
>
> Cette vie aseptique n'entraîne par elle-même aucune déchéance de l'organisme.

Kianigin ('17) has recently reopened this question by a review of all the literature. At first sight it appears incomprehensible that aseptic life should be so difficult when the greatest quantity of microörganisms is located in the non-digestive portions of the digestive tract. Metchnikoff ('09) points out, however, that the digestive powers of newly born are much weaker than those of older animals. The increasing number of cases of organisms which can be raised aseptically indicates that an aseptic existence may be possible in the majority of cases.

Nencki ('86) gave indirect evidence in this direction when he showed that the action of digestive ferments on food-stuffs makes them very quickly soluble and absorbable. The action of bacteria merely carries the decomposition to a lower level, yielding unassimilable aromatic acids, fatty acids, phenol, kresol, indol, skatol, carbon dioxide, methan, etc. The indications are, however, that microörganisms are of value as intestinal flora not because of their digestive, but because of their synthetic power.

MICROÖRGANISMS AS INTERNAL SYMBIONTS OF INSECTS

In addition to the cases of internal symbiosis of fungi with insects inhabiting dry wood, many Hemiptera and Blattidæ are also associated with microörganisms. These are usually bacteria or yeasts and infest the ovary. For example, the pseudovitellus of the aphid was long a puzzle to embryologists, but finally proved to be a granular body containing yeast cells which in the further development of the insect make complicated migrations and finally become lodged in certain fat-bodies which are termed mycetocytes or bacteriocytes, after which the infection of the ovary takes place. The evidence that a real symbiotic relationship exists in the Blattidæ and Hemiptera is given by Buchner and others as follows:

1. All eggs are infected.
2. The infection is not injurious to them.
3. Each species of insect is associated with a definite species of microörganism.
4. This association is very definite and almost a specific character.
5. The yeasts profit by the relationship in the protection which they receive from the host against the vicissitudes of the environment.
6. The yeasts multiply as the animal multiplies, always being present in constant amount even in such rapidly increasing forms as aphids and coccids.
7. The yeasts and bacteria are of value to the host as destroyers of waste products of metabolism, such as urates, according to

Sulc ('10b), and as absorbers of excess food materials such as sugar, according to Pierantoni ('10).

The movements and location of the symbionts has been studied by Buchner ('12) in a great work on the Blattidæ. Glasgow ('14) has also studied a case of symbiosis in which the microörganism, instead of being located in a fat-body (mycetocytes), as in the cockroaches, is retained in very highly developed gastric caeca of the plant bugs (Heteroptera). The function ascribed to the bacteria by Glasgow is the prevention of infection of the digestive tract by other bacteria. Dissected digestive tracts on bacterial media gave only pure growths of the associated microörganisms. Petri ('04, '05, '06) studied the similar case of Dacus oleae, which feeds on the olive, but ascribes to the bacillus alipolytic enzyme of assistance in the digestion of the food. Schaudinn ('04) finds that a fungus (Entomophthorineae) is transmitted through the egg of Culex and is always found in the diverticulae of the oesophagus of adult Anopheles and Culex. He has been able to rear the fungus in sugar solution and has demonstrated that CO_2 is formed in the imago from the sugars in the blood which it has sucked up. It is probable that the irritation caused by the bite of mosquitoes is largely due to enzymes secreted by these fungi.

In these cases the associated fungus may be a commensal, in its relation to the host, profiting by an oversupply of some food substance, as in the case of aphids, mosquitoes, etc., or may be of value as a chemical agent, as in the case of Dacus oleae, or may be of service in maintaining an unchanged digestive flora, as in the Heteroptera, as described by Glasgow. In general, however, the exact function of the microörganism to its host has not been thoroughly explained.

CONCLUSION

I have shown by experiments that Drosophila living in fermenting fruit are dependent for their food supply on the synthetic and absorptive powers of yeast cells. In a similar manner, my study of the relation of Musca domestica to manure, of Desmometopa to decaying meat, and of Sciara and Tyroglyphus to decaying

wood shows clearly that these Arthropods also feed on microörganisms. I have also endeavored to account for the origin and devolopment of this habit, to ascertain the probable extent of its occurrence, and to consider the known associations of animals with fungi in general. The experiments and considerations all tend to establish the principle that insects inhabiting fermenting and decaying substrata of low protein content, usually feed upon the microörganisms present and thus benefit by the power of the fungi to extract, adsorb, and synthesize many non-protein nitrogenous compounds.

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Resumido por los autores, H. D. Goodale y Grace MacMullen.

La importancia de los índices en las teorías sobre la herencia de la producción de huevos de invierno.

Los índices requeridos por la teoría mendeliana de la herencia de la fecundidad, desarrollados por Pearl, coinciden con los observados en una larga serie de datos obtenidos en la Estación Experimental de Agricultura de Massachusetts. Por otra parte, los autores demuestran que los índices observados y los obtenidos teóricamente coinciden también si se admite que la fecundidad elevada depende de dos factores, cualquiera de los cuales sin el otro, da lugar a una producción mediocre, y cuya herencia sigue el esquema ordinario en los dihíbridos. La coincidencia entre los datos observados y los teóricos es independiente de cualquier punto de división particular entre los individuos que producen abundantes huevos y los que producen una cantidad mediocre y parece depender del pequeño número de individuos de las familias individuales. Ninguna teoría, por esta causa, se apoya en una base firme garantizada por los hechos. En el presente trabajo se incluyen datos que demuestran que la fecundidad elevada no se transmite como un carácter ligado al sexo. No se excluye la posibilidad de que la cantidad de producción esté determinada por uno o varios factores ligados al sexo. Parece probable que el número de huevos no se hereda coma una unidad sino que es el resultado de varias unidades que pueden heredarse.

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THE BEARING OF RATIOS ON THEORIES OF THE INHERITANCE OF WINTER EGG PRODUCTION

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INTRODUCTION

The problem

We owe to Pearl ('12) the first Mendelian theory applicable to the inheritance of fecundity. This theory may be stated as follows. The winter egg production of the pullet year, i.e., the eggs laid before March 1, is a good index of annual production. Production during this period falls into three classes, viz., high

(over 30 eggs),[1] mediocre (under 30 eggs), and zero. The ratios in which these three classes occur in the various families can be accounted for, if it is assumed that there are two factors (designated L_1 and L_2 by Pearl) additional to the factor common to all pullets which determines that they shall lay at all. The L_1 factor follows the simple monohybrid, Mendelian scheme of inheritance, the L_2 factor that of a sex-linked factor. It is further assumed that absence of both factors results in zero winter production, and that one factor alone ,either in single or double dose, gives mediocre production, while both must be present to give high winter laying.

Although in the great majority of cases the observed ratios agree with the expected ratios, Pearl encounters two sorts of difficulties. First, the mediocre and zero classes often contain more than their expected share of the progeny. This is natural, for it is obvious to anyone at all familiar with poultry that birds carrying genes for a given degree of production may fail to reach that production. The second difficulty that Pearl encounters is that birds theoretically lacking the proper gametic constitution, nevertheless make records that cannot be distinguished from those that theoretically have the gametic constitution for high production. The explanation usually offered for these instances is that they are 'somatic variations.' Pearl means, of course, that there is some overlapping between the two systems of phaenotypes. This occurs through an excess of birds in the high class and is most easily observed when the expected number of birds is zero.

While the agreement between the observed and expected ratios is satisfactory, the question arises as to whether or not this agreement alone is to be regarded as sufficient proof of the validity of the theory. May there not be other conditions that must be satisfied? Are there shortcomings in the observed data of such a character that their value is reduced? May there not be another theory (or theories) that fit the observed data? We shall show that these questions are answered in the affirmative.

[1] We adopt the convention for our data of calling birds laying 30 or more eggs as 'over 30,' but those laying 29 or less are classified as 'under 30' or zero.

An alternative theory

In working over this problem, another theory has been found, whose ratios fit the observed ratios[2] equally as well as that proposed by Pearl. In addition it has the following advantages:

1. It is somewhat simpler since it does not involve sex linkage.
2. It accounts genetically for the birds in the over-30 class for which Pearl's theory requires a supplementary explanation.
3. The only marked departures from the expected ratios are downward, *i.e.*, there is a deficiency of high producers. This deficiency can be accounted for physiologically.

The alternative theory may be stated in this form. Winter egg production falls into two classes, viz., high (over 30), mediocre (under 30). The observed ratios agree with those expected if it be assumed that high fecundity depends upon the simultaneous presence of two factors, A and B, in the zygote, while mediocre production depends upon the presence of not more than one of these two factors in duplex, simplex, or nulliplex condition. The two factors are inherited according to the usual dihybrid scheme. The theoretical ratios resulting are shown in table 1.[3] It will be noted that several classes of males cannot be distinguished from each other by the progeny test alone. Thus class 2 cannot be distinguished from 5, 4 from 8 and 6 from 7. The members of each pair can be distinguished from each other only by the parental test.

The ratios expected on Pearl's theory are given in table 2 (modified from Pearl, '12).

The detailed reasons for grouping the zero class with the mediocre producers have been discussed in another place (Goodale, '18) and need not be repeated here. It will be sufficient to

[2] The observed ratios cannot be explained by assuming a single factorial difference between high and mediocre producers. Several more complicated schemes, however, can be devised that furnish the desired theoretical ratios.

[3] The use of the form of the presence and absence theory does not necessarily imply an acceptance of the view that material particles are present or absent. Whenever a change takes place in a locus of a chromosome, it is clear that an absence of the preceding condition occurs. Thus, A represents merely a condition of a chromosome (or locus) different from a.

TABLE 1

Ratios and classes expected on the alternative theory. The classes are printed in italics

			Males								
	CLASS	CLASS	I	II	III	IV	V	VI	VII	VIII	IX
		Gametic constitution	AA BB	AaBB	AaBb	Aabb	AABb	AAbb	aaBB	aaBb	aabb
Females	1	AABB	16 : 0 *1*	16 : 0 *1, 2*	16 : 0 *1, 2, 3, 5*	16 : 0 *3, 5*	16 : 0 *1, 5*	16 : 0 *5*	16 : 0 *2*	16 : 0 *2, 3*	16 : 0 *3*
	2	AaBB	16 : 0 *1, 2*	12 : 4 1, *2, 7*	12 : 4 *1, 2, 3, 5* *7, 8*	12 : 4 *3, 5, 8*	16 : 0 *1, 2, 3, 5*	16 : 0 *3, 5*	8 : 8 *2, 7*	8 : 8 *2, 3, 7, 8*	8 : 8 *3, 9*
	3	AaBb	16 : 0 *1, 2, 3, 5*	12 : 4 *1, 2, 3, 5* *7, 8*	9 : 7 *1, 2, 3, 4* *5, 6, 7* *8, 9*	6 : 10 *3, 4, 5, 6* *8, 9*	12 : 4 *1, 2, 3, 4* *5, 6*	8 : 8 *3, 4, 5, 6*	8 : 8 *2, 3, 7, 8*	6 : 10 *2, 3, 4, 7* *8, 9*	4 : 12 *3, 4, 8, 9*
	4	Aabb	16 : 0 *3, 5*	12 : 4 *3, 5, 8*	6 : 10 *3, 4, 5, 6* *8, 9*	0 : 16 *4, 6, 9*	8 : 8 *3, 4, 5, 6*	0 : 16 *4, 6*	8 : 8 *3, 8*	4 : 12 *3, 4, 8, 9*	0 : 16 *4, 9*
	5	AABb	16 : 0 *1, 5*	16 : 0 *1, 2, 3, 5*	12 : 4 *1, 2, 3, 4* *5, 6*	8 : 8 *3, 4, 5, 6*	12 : 4 *1, 5, 6*	8 : 8 *5, 6*	16 : 0 *2, 3*	12 : 4 *2, 3, 4*	8 : 8 *3, 4*
	6	AAbb	16 : 0 *5*	16 : 0 *3, 5*	8 : 8 *3, 4, 5, 6*	0 : 16 *4, 6*	8 : 8 *5, 6*	0 : 16 *6*	16 : 0 *3*	8 : 8 *3, 4*	0 : 16 *4*

Females.	7	aaBB	16:0 *2*	8:8 *2, 7*	8:8 *2, 3, 7, 8*	8:8 *3, 8*	16:0 *2, 3*	0:16 *3*	0:16 *7*	0:16 *7, 8*	0:16 *8*
	8	aaBb	16:0 *2, 3*	8:8 *2, 3, 7, 8*	6:10 *2, 3, 4, 7 8, 9*	4:12 *3, 4, 8, 9*	12:4 *2, 3, 4*	8:8 *3, 4*	0:16 *7, 8*	0:16 *7, 8, 9*	0:16 *8, 9*
	9	aabb	16:0 *3*	8:8 *3, 8*	4:12 *3, 4, 8, 9*	0:16 *4, 9*	8:8 *3, 4*	0:16 *4*	0:16 *8*	0:16 *8, 9*	0:16 *9*
Totals			144:0	108:36	81:63	54:90	108:36	72:72	72:72	54:90	36:108
Ratios			1:0	3:1	9:7	3:5	3:1	1:1	1:1	3:5	1:3

TABLE 2

Ratios expected on Pearl's theory (modified from Pearl, '12).

Males

	CLASS	CLASS	I	II	III	IV	V	VI	VII	VIII	IX
	CLASS	Gametic constitution	$fL_1L_2fL_1L_2$	$fL_1L_2fL_1l_2$	$fL_1L_2fl_1L_2$	$fL_1L_2fl_1l_2$	$fL_1l_2fL_1l_2$	$fL_1l_2fl_1l_2$	$fl_1L_2fl_1L_2$	$fl_1L_2fl_1l_2$	$fl_1l_2fl_1l_2$
Females	1	$fL_1L_2.Fl_1l_2$	16:0:0	12:12:0	18:6:0	9:12:3	0:16:0	0:18:6	12:12:0	6:12:6	0:12:12
	2	$fL_1L_2.FL_1l_2$	16:0:0	6:6:0	12:0:0	6:6:0	0:16:0	0:12:0	12:0:0	6:6:0	0:12:0
	3	$fL_1l_2.Fl_1l_2$	16:0:0	12:12:0	18:6:0	9:12:3	0:16:0	0:18:6	12:12:0	6:12:6	0:12:12
	4	$fL_1l_2.FL_1l_2$	16:0:0	6:6:0	12:0:0	6:6:0	0:16:0	0:12:0	12:0:0	6:6:0	0:12:0
	5	$fl_1l_2.Fl_1l_2$	16:0:0	6:6:0	6:6:0	3:6:3	0:16:0	0:6:6	0:12:0	0:6:6	0:0:12
	6	$fl_1L_2.Fl_1l_2$	16:0:0	6:6:0	6:6:0	3:6:3	0:16:0	0:6:6	0:12:0	0:6:6	0:0:12
Totals			96:0:0	48:48:0	72:24:0	36:48:12	0:96:0	0:72:24	48:48:0	24:48:24	0:48:48
Ratios			1:0:0	1:1:0	3:1:0	3:4:1	0:1:0	0:3:1	1:1:0	1:2:1	0:1:1

recall that the evidence favors the view that this class is wholly artificial and depends for its existence upon an artificial division point in the calendar year. However, no difficulties need be expected in fitting the observed to expected ratios if the alternative theory be modified to consist of Pearl's three classes, for it can be assumed that the double nulliplex class accounts for the zero producers. We have applied this modification to several of the observed ratios and found that it fitted well.

THE MATERIAL AND ITS TREATMENT

The kind and sources of data used

The observations presented in this paper are the winter egg records of our Rhode Island Red pullets for the years 1913 to 1917, inclusive, together with Pearl's data on Barred Plymouth Rocks, pure Cornish, and the crosses between the two breeds. (Pearl, '12). We have also a small amount of data on pure Cornish and one cross of these with the Rhode Island Reds, the latter, however, of prime importance.

The arrangement of data

The data are presented in the following order:

1) A table (no. 4) showing Pearl's observed and expected ratios compared with those of the alternative theory. 2) A table (no. 5) showing the observed and expected ratios of our own data arranged according to the two theories, together with such comment as seems necessary.

The primary object of this paper is to test the applicability of Pearl's theory to our data. Consequently, we have reduced our data to essentially the same form employed by Pearl. Moreover, we have included in our data only birds hatched in April and May in order to conform to Pearl's practices in this respect.

In this study we have limited ourselves to a consideration of one phase of the problem of the inheritance of winter egg production. Later we purpose to present our data in a form that

will make them available to all and at that time to consider the subject from several other angles, among them that of selection. We may anticipate the proposed work sufficiently to note that egg production yields readily to selection on a suitable basis, as Pearl ('15 a and b) and Dryden ('16) have already found.

The position of the numerical division point between high and mediocre producers

The determination of a suitable numerical division point has been a matter of concern in our work, for it represents the line of separation between two genotypes, which, however, may overlap. The obvious method of finding this point is to plot a distribution curve of the winter egg production, and determine the point at which the antimode occurs. This method indicates no other division point for our Rhode Island Reds except the one at or near 30 eggs. At the same time the evidence is not of a very striking character. Further, I have shown that the time of hatching has a marked influence on the shape of these curves (Goodale, '18). We have, therefore, assumed other division points and worked out a comparison between the observed and theoretical ratios which is given in table 5.

A division point at 40 eggs has some evidence in its favor. An inspection of our records indicates that February 15 corresponds more closely to the end of the winter period than March 1, because during the last half of February there is a resumption of production on the part of birds that have undergone a winter pause. Moreover, the curve of daily egg production shows a sharp upward turn at this time. The rate of production of birds at this season is sufficiently high to make the last fourteen days of February yield 9 or 10 eggs, so that an egg production of 40 eggs before March 1 is the equivalent of 30 eggs laid before February 15. Because of the form in which the records are transcribed, it is much simpler to handle the data with a division point at 40 eggs laid before March 1 than to use a division point at 30 eggs laid before February 15. There is no objection to this method of treating the data, for the very few birds that

begin to lay for the first time between February 14 and March 1 may be easily sorted out and classified as zero instead of mediocre producers. We have not done this, however, in this paper as they are too few to affect the ratios seriously.

The results of assuming various division points are these. All division points tried give ratios that fit the observed equally well (compare table 5). The gametic classes in which the individual breeders fall, however, shift with the change. For example, in 1917–18, the various males belong to high-producing classes with a division point at 30 eggs, but to low classes if the division point is made at 60 eggs.

DATA ON BARRED PLYMOUTH ROCKS, CORNISH, AND RECIPROCAL CROSSES

Although the application of the alternative theory to Pearl's data, table 4, has ordinarily been made without difficulty, in some instances, the fact that the data are grouped to fit a different scheme has made it impossible to secure a good fit. An example is given by male no. 558 which was mated to five females, all referred by Pearl to Cornish females of class 1. They give the ratio of 1 high to 9 mediocre producers. Although the 1:9 ratio cannot be accounted for, it is clear that one female produced one high and either one, two, three, or four mediocre daughters, the other females producing mediocre daughters only. Such ratios are accounted for by the alternative theory. The other cases of poor fit are doubtless of the same character.

In several instances it is possible to refer a male to more than one gametic class, but this is not done unless the ratios are identical.

When Pearl modifies the ratios to accord with physiological facts, the modified ratio is used, except when the modification made does not affect the alternative theory, e.g., when some zero producers are shown to be mediocre producers. In two instances, viz., males no. 26 and no. 557, where the alternative theory does not fit the observed ratios as well as Pearl's theory, the deviations are not beyond the bounds of probability. Moreover, the deviation is in the direction that might be expected

from a physiological character like egg production, viz., there is a deficiency in the group of high producers, that is, the genes for high production may be present, but do not come to expression. This failure may be due to other genetic causes, such as a tendency to slow development or subnormal vitality. But whatever the explanation, it is clear, on the alternative theory, that all Cornish males by Barred Plymouth Rock females show a slight deficiency of high producers.

In the case of male no. 578 mated with barred F_1 females (Pearl, '12, p. 373), the alternative theory gives ratios that fit the observed ratios, though this instance does not conform to Pearl's theory, as he himself recognizes.

The results of mating male no. 578 to black F_1 females can be explained on the alternative theory as was done for male no. 558.

In table 31 (Pearl's) the grouping of the parents precludes an application of the alternative theory.

DATA ON RHODE ISLAND REDS AND CORNISH

General

In table 5 is given a comparison between the observed (Roman type) and expected (italics) ratios arranged according to the two theories under consideration and the various division points assumed. The mean winter egg production of the various groups is also given under the heading 'Pearl's theory' in black-faced type.

The records made in the winter of 1913–14 are those of pullets from non-pedigreed stock. The succeeding years represent mainly succeeding generations descended from this stock. Considerable new blood, however was added in 1915. As any number is assigned to only one individual, the reader can readily ascertain which males were used in more than one breeding season. With the exception of 1914–15, males have always been assigned the same gametic constitution. The egg production for 1914–15 is clearly abnormal, nevertheless, by not attempting to assign the same gametic constitution as in other

TABLE 3

Representative Rhode Island Red families arranged to show a comparison between the observed and expected ratios of the offspring of individual mothers. All ratios in table 5 are obtained by this method

FATHER'S BAND NUMBER	MOTHER'S BAND NUMBER	PEARL'S THEORY								ALTERNATIVE THEORY					
		Class of father	Class of mother	Observed ratio			Expected ratio			Class of father	Class of mother	Observed ratio		Expected ratio	
				30 and over	Under 30	Zero	30 and over	Under 30	Zero			30 and over	Under 30	30 and over	Under 30
6373	3600	III	6	5	1	0	3	3	0	IV	7	5	1	3	3
	174		6	2	2	0	2	2	0		1	2	2	4	0
	284		2	3	1	0	4	0	0		1	3	1	4	0
	4778		1	2	1	0	2.25	0.75	0		5	2	1	1.50	1.50
Totals				*12*	*5*	*0*	*11.25*	*5.75*	*0*			*12*	*5*	*12.50*	*4.50*
3617	5230	III	1	2	1	0	2.25	0.75	0	II	2, 3	2	1	2.25	0.75
	6751		2	7	0	0	7	0	0		1	7	0	7	0
	4409		2	5	0	0	5	0	0		5	5	0	5	0
	4716		2	10	0	0	10	0	0		5	10	0	10	0
	6003		1	6	4	0	7.50	2.50	0		2, 3	6	4	7.50	2.50
Totals				*30*	*5*	*0*	*31.75*	*3.25*	*0*			*30*	*5*	*31.75*	*3.25*
5584	5106	VIII	1	3	5	3	2.75	5.50	2.75	IX	3	3	8	2.75	8.25
	5290		1	0	3	3	1.50	3.00	1.50		3	0	6	1.50	4.50
	6067		1	0	4	7	2.75	5.50	2.75		3	0	11	2.75	8.25
Totals				*3*	*12*	*13*	*7.00*	*14.00*	*7.00*			*3*	*25*	*7.00*	*21.00*

4378	4043	IV	1	1	4	2	2.63	3.50	0.87	VIII	3	1	6	2.63	4.37
	4754		3	0	5	1	2.25	3.00	0.75		7, 8, 9	0	6	0	6
	4012		6	6	4	2	3	6	3		6	6	6	6	6
	4592		6	0	1	3	1	2	1		4	0	4	1	3
Totals				*7*	*14*	*8*	*8.88*	*14.50*	*5.62*			*7*	*22*	*9.63*	*19.37*
4723	4178	III	6	5	4	1	5	5	0	III	7	5	5	5	5
	4845		2	11	1	0	12	0	0		1	11	1	12	0
	4138		1	9	3	0	9	3	0		2	9	3	9	3
	4473		1	9	6	1	12	4	0		3	9	7	9	7
	5476		1	8	2	0	7.50	2.50	0		2, 5	8	2	7.50	2.50
	5463		1	5	2	0	5.25	1.75	0		2, 5	5	2	5.25	1.75
Totals				*47*	*18*	*2*	*50.75*	*16.25*	*0*			*47*	*20*	*47.75*	*19.25*
5477	4844	I	1, 2	11	0	0	11	0	0	Several classes	Several classes	11	0	11	0
	6366		1, 2	4	0	0	4	0	0			4	0	4	0
Totals				*15*	*0*	*0*	*15*	*0*	*0*			*15*	*0*	*15*	*0*

TABLE 4

Ratios, observed and expected, based on both Pearl's theory and the alternative theory. Data taken from Pearl, '12

FATHER'S NUMBER	NUMBER OF MOTHERS	PEARL'S THEORY						ALTERNATIVE THEORY			
		Observed ratio			Expected ratio			Observed ratio		Expected ratio	
		Over 30	Under 30	Zero	30 and over	Under 30	Zero	Over 30	Under 30	Over 30	Under 30
Barred Plymouth Rock matings											
553	11	25	9	0	26.50	7.50	0	25	9	26.50	7.50
		50.48	**13.56**	**0**							
567	10	21	15	0	22.50	13.50	0	21	5	21.62	14.38
		55.95	**15.64**	**0**							
562	6	13	6	0	13.50	5.50	0	13	6	13.50	5.50
		59.38	**11.67**	**0**							
552	17	30.50	16.50	2	30.50	18.50	0	30.50	18.50	30.50	18.50
		51.07	**13.06**	**0**							
554	13	20	15	1	20.50	15.50	0	20	16	20.50	15.50
		52.20	**16.53**	**0**							
564	10	11	14	2	12.50	14.50	0	11	16	9.87	17.13
		62.64	**18.85**	**0**							
D58	13	10	18	1	12	17	0	10	19	9	20
		52.22	**16.82**	**0**							
Cornish Indian Game Matings											
558	7	1	14	3	0	16	2	1	17	0	18
		37	**8.36**	**0**							
578	5	1	9	12	0	9	13	1	21	0	22
		39	**13.11**	**0**							
Barred Plymouth Rock ♂♂ by Cornish ♀♀											
559	10	23	18	2	24.50	18.50	0	23	20	24.50	18.50
		46.09	**15.22**	**0**							
554	1	1	3	0	2	2	0	1	3	2	2
		49	**19.33**	**0**							
Cornish ♂♂ by Barred Plymouth Rock ♀♀											
558	3	4	17	3	0	18	6	4	20	5	19
		51.75	**14.06**	**0**							
557	7	0	18	0	0	18	0	0	18	4.25	13.75
		0	**12.72**	**0**							

573	8	10.50 : 7.50 : 1 **49.80 15.71 0**	11.50 : 7.50 : 0	10.50 : 8.50	10.13 : 8.87	
56	11	16 : 7 : 0 **55.87 17 0**	13.50 : 9.50 : 0	16 : 7	15 : 8	
563	11	29 : 12 : 1 **63.76 16.50 0**	30.50 : 11.50 : 0	29 : 13	30.50 : 11.50	
D31	26	57 : 29 : 2 **48.16 13.81 0**	62 : 26 : 0	57 : 31	56.25 : 31.75	
569	12	12.50 : 15.50 : 4 **53.58 7.60 0**	11.65 : 16 : 4.35	12.50 : 19.50	12.63 : 19.37	
566	11	13 : 14 : 2 **45.69 16.79 0**	12.70 : 14.50 : 1.80	13 : 16	11 : 18	
D35	7	7 : 12 : 1 **47.43 16.25 0**	8 : 10 : 2	7 : 13	6.13 : 13.87	
556	13	19 : 21 : 4 **46 19.60 0**	19.10 : 22 2 : 90	19 : 25	22 : 22	
65	2	9 : 4 : 1 **49.44 18.75 0**	9.25 : 4.75 : 0	9 : 5	9.25 : 4.75	
68	4	13 : 5 : 2 **59 25 0**	15 : 5 : 0	13 : 7	15 : 5	
529	10	7 : 16 : 3 **42.71 12 0**	0 : 22.25 : 3.75	7 : 19	9 : 17	
578	3	1 : 7 : 0 **42 9 0**	0 : 8 : 0	1 : 7	1.75 : 6.25	

Matings of the second cross-bred (F_2) generation.

576	7	11 : 20 : 6 **42.81 12.05 0**	13.90 : 18.50 : 4.60	11 : 26	14 : 23	
577	5	5 : 16 : 7 **36.33 11.25 0**	7 : 14 : 7	5 : 23	6.87 : 21.13	
576	3	9.50 : 6.50 : 0 **46.78 22.83 0**	8 : 8 : 0	9.50 : 6.50	10.13 : 5.87	
557	3	2.50 : 4.50 : 0 **35 11.25 0**	3.50 : 3.50 : 0	2.50 : 4.50	2.63 : 4.37	
577	2	0 : 3 : 2 **0 7.33 0**	1.25 : 2.50 : 1.25	0 : 5	0 : 5	
552	1					
554	1					
563	1	19.50 : 13.50 : 3 **57.16 12.69 0**	18 : 18 : 0	19.50 : 16.50	19.87 : 16.13	
564	1					
567	1					
562	1					

TABLE 4—*Continued*

Father's number	Number of mothers	Pearl's theory: Observed ratio: Over 30	Under 30	Zero	Pearl's theory: Expected ratio: 30 and over	Under 30	Zero	Alternative theory: Observed ratio: Over 30	Under 30	Alternative theory: Expected ratio: Over 30	Under 30
Barred Plymouth Rock matings—*Continued*											
32	2	4	3	0	3.50	3.50	0	4	3	3.50	3.50
		52.50	**14.33**	**0**							
57	4	6	4	0	5	5	0	6	4	6.19	3.81
		54.50	**23.25**	**0**							
17	2	3	3	0	3	3	0	3	3	3	3
		47	**12.33**	**0**							
70	5	6	6	0	6	6	0	6	6	6	6
		62.67	**20**	**0**							
26	7	8.50	15.50	5	10.25	14.50	4.25	8.50	20.50	12.37	16.63
		60.50	**12.26**	**0**							
550	10	16.50	1.50	0	18	0	0	16.50	1.50	18	0
		51.25	**1**	**0**							
Matings of the second cross-bred (F) generation—*Continued*											
566	1	2	3	1	2.25	3.75	0	2	4	2.25	3.75
		46.50	**13.67**	**0**							
578	3	1	9	1	0	11	0	1	10	2.75	8.25
		41	**9.67**	**0**							
578	4	9.50	27.50	0	?			9.50	27.50	12	25
		45.67	**16.52**	**0**							

years, a good fit between observed and expected ratios is obtained, although such a result is hardly to be expected. Moreover, the data for 1914–15, if combined with that of whatever other year may be involved, give satisfactory ratios except for one male, viz., male no. 271. The ratios for this male are, on Pearl's theory, 3:32:12 observed, to 0:35.50:11.50 expected. On the alternative theory the ratios are 3:44 observed, to 11.50:35.50 expected.

The various years are not wholly comparable to one another. Certain changes in management, described in later sections, have been forced upon us. Selective matings have also been made in various years. Some were made for high production, but others were made primarily for low production, late maturity, hatching quality of eggs, vigor, broodiness, and size. Thus, a portion of the matings each year are made at random as far as egg production is concerned.

In compiling these tables the progeny of each pair was first distributed into the three (respectively two) groups of producers required by each theory and the expected ratio that agreed most closely with the observed ratio determined by inspection (compare table 3). It was of course necessary to select a gametic constitution for the male that would give suitable ratios for all the females with which he was mated.

An exact fit for all females cannot be expected, but in only two instances has the deviation been greater than three and one-half individuals, i.e., a change of three individuals and one-half from one class to another makes the observed and expected ratios agree perfectly. Changes of three or three and one-half individuals are rare. The sum of the observed and expected ratios of the various mates of each male constitute the data given in table 5.

Within the limits designated, we have not hesitated to choose that theoretical ratio which gives the closest fit in the total. In some instances it is possible to assign more than one theoretical ratio, but we have not done so.

By accepting the discrepancies mentioned between observed and expected ratios, we avoid classifying an individual breeder

TABLE 5

Ratios, observed and expected, based on both Pearl's theory and the alternative theory and with several division points between high and mediocre producers. Observed ratios in Roman, expected in Italics. Mean egg production for each group in black-faced type. Rhode Island Reds, unless otherwise stated

FATHER'S BAND NUMBER	NUMBER OF MOTHERS	PEARL'S THEORY						ALTERNATIVE THEORY			
		Division point in eggs						Division point in eggs			
		30			40			30		40	
		30 and over	Under 30	Zero	40 and over	Under 40	Zero	30 and over	Under 30	40 and over	Under 40
1913–1914											
A310	7	14 : 6 : 1			6 : 14 : 1			14 : 7		6 : 15	
		15 : 6 : 0			*7.50 : 13.50 : 0*			*14.50 : 6.50*		*7 : 14*	
		40.86	**19.50**	**0**	**49.17**	**28.14**	**0**				
A312	4	7 : 2 : 0			5 : 4 : 0			7 : 2		5 : 4	
		6 : 3 : 0			*6 : 3 : 0*			*7.50 : 1.50*		*4.50 : 4.50*	
		43.57	**12**	**0**	**42.20**	**24.50**	**0**				
A313	3	2 : 6 : 0			2 : 6 : 0			2 : 6		2 : 6	
		4 : 4 : 0			*4 : 4 : 0*			*3 : 5*		*3 : 5*	
		44.50	**16.67**	**0**	**44.50**	**16.67**	**0**				
A316	6	5 : 6 : 1			4 : 7 : 1			5 : 7		4 : 8	
		5.13 : 6 : 0.87			*6 : 6 : 0*			*6 : 6*		*3.75 : 8.25*	
		46	**17.67**	**0**	**48.25**	**20.43**	**0**				
A143	4	4 : 2 : 0			2 : 4 : 0			4 : 2		2 : 4	
		3 : 3 : 0			*2.50 : 3 : 0.50*			*3.63 : 2.37*		*3 : 3*	
		44	**20.50**	**0**	**53.50**	**27.50**	**0**				
A302	2	7 : 0 : 1			5 : 2 : 1			7 : 1		5 : 3	
		8 : 0 : 0			*5 : 3 : 0*			*5.50 : 2.50*		*4 : 4*	
		50.86	**0**	**0**	**56.60**	**36.50**	**0**				
A305	6	16 : 2 : 0			14 : 4 : 0			16 : 2		14 : 4	
		15.50 : 2.50 : 0			*14.75 : 3.25 : 0*			*16.75 : 1.25*		*14 : 4*	
		54.44	**22.50**	**0**	**56.93**	**29.75**	**0**				
A307	5	6 : 2 : 0			2 : 6 : 0			6 : 2		2 : 6	
		7 : 1 : 0			*3 : 5 : 0*			*6 : 2*		*2.50 : 5.50*	
		37	**14**	**0**	**46**	**26.33**	**0**				

A323	6	12 : 4 : 1 *13 : 4 : 0* **53.25 11.75 0**	8 : 8 : 1 *7.37 : 8.50 : 1.13* **63.13 22.63 0**	12 : 5 *11.50 : 5.50*	8 : 9 *7.13 : 9.87*	A308	5	16 : 6 : 0 *15.50 : 6.50 : 0* **45.38 19 0**	12 : 10 : 0 *14.50 : 7.50 : 0* **49.25 24.90 0**	16 : 6 *15.75 : 6.25*	12 : 10 *12 : 10*
A114	7	8 : 2 : 0 *8 : 2 : 0* **47.13 14 0**	6 : 4 : 0 *6 : 4 : 0* **51.50 24 0**	8 : 2 *8 : 2*	6 : 4 *6 : 4*	A324	4	6 : 4 : 0 *5.50 : 4.50 : 0* **40.17 11 0**	2 : 8 : 0 *2 : 8 : 0* **51.50 22.50 0**	6 : 4 *6.50 : 3.50*	2 : 8 *2 : 8*

1914–1915

807	2	3 : 3 : 1 *3.50 : 3.50 : 0* **45 10 0**	2 : 4 : 1 *3.50 : 3.50 : 0* **50 16.25 0**	3 : 4 *3 : 4*	2 : 5 *1.75 : 5.25*	17	2	0 : 4 : 0 *0 : 4 : 0* **0 8.75 0**	0 : 4 : 0 *0 : 4 : 0* **0 8.75 0**	0 : 4 *0.75 : 3.25*	0 : 4 *0.75 : 3.25*
A291	2	2 : 2 : 0 *3 : 1 : 0* **35 14 0**	1 : 3 : 0 *2 : 2 : 0* **40 18.67 0**	2 : 2 *2 : 2*	1 : 3 *1 : 3*	250	6	0 : 9 : 5 *0 : 10.75 : 3.25* **0 8.33 0**	0 : 9 : 5 *0 : 10.75 : 3.25* **0 8.33 0**	0 : 14 *0.75 : 13.25*	0 : 14 *0.75 : 13.25*
1480	1	0 : 1 : 0 *0 : 1 : 0* **0 18 0**	0 : 1 : 0 *0 : 1 : 0* **0 18 0**	0 : 1 *0.25 : 0.75*	0 : 1 *0 : 1*	283	3	0 : 5 : 2 *0 : 5.75 : 1.25* **0 7 0**	0 : 5 : 2 *0 : 5.75 : 1.25* **0 7 0**	0 : 7 *1.50 : 5.50*	0 : 7 *1.25 : 5.75*
389	1	0 : 1 : 0 *0 : 1 : 0* **0 19 0**	0 : 1 : 0 *0 : 1 : 0* **0 19 0**	0 : 1 *0 : 1*	0 : 1 *0 : 1*	228	2	1 : 5 : 1 *0 : 5.50 : 1.50* **38 17.40 0**	0 : 6 : 1 *0 : 7 : 0* **0 20.83 0**	1 : 6 *1.75 : 5.25*	0 : 7 *1.75 : 5.25*
A290	4	0 : 10 : 4 *0 : 11.75 : 2.25* **0 9.80 0**	0 : 10 : 4 *0 : 11 : 3* **0 9.80 0**	0 : 14 *1.75 : 12.25*	0 : 14 *1 : 13*	337	4	2 : 8 : 2 *4.13 : 6 : 1.87* **42.50 7.86 0**	0 : 10 : 2 *0 : 9 : 3* **52 11 0**	2 : 10 *3 : 9*	0 : 12 *1.50 : 10.50*
619	2	4 : 3 : 0 *3.50 : 3.50 : 0* **40.25 11 0**	3 : 4 : 0 *3.50 : 3.50 : 0* **43 18 0**	4 : 3 *3.25 : 3.75*	3 : 4 *3.25 : 3.75*	A323	5	0 : 13 : 0 *0 : 13 : 0* **0 13.62 0**	0 : 13 : 0 *0 : 13 : 0* **0 13.62 0**	0 : 13 *3.25 : 9.75*	0 : 13 *1.50 : 11.50*

TABLE 5—*Continued*

FATHER'S BAND NUMBER	NUMBER OF MOTHERS	PEARL'S THEORY						ALTERNATIVE THEORY			
		Division point in eggs						Division point in eggs			
		30			40			30		40	
		30 and over	Under 30	Zero	40 and over	Under 40	Zero	30 and over	Under 30	40 and over	Under 40
804	3	1 : 7 : 1 *2.25 : 4.50 : 2.25* **42 9.43 0**			1 : 7 : 1 *0 : 6.50 : 2.50* **42 9.43 0**			1 : 8 *2.75 : 6.25*		1 : 8 *1.87 : 7.13*	
2269	2	0 : 4 : 1 *0 : 5 : 0* **0 20.50 0**			0 : 4 : 1 *0 : 4 : 1* **0 20.50 0**			0 : 5 *1.25 : 3.75*		0 : 5	
227	2	1 : 2 : 0 *1.50 : 1.50 : 0* **46 18.50 0**			1 : 2 : 0 *1 : 2 : 0* **46 18.50 0**			1 : 2 *1.25 : 1.75*		1 : 2 *1.25 : 1.75*	
525	1	0 : 1 : 0 *0 : 1 : 0* **0 1 0**			0 : 1 : 0 *0 : 1 : 0* **0 1 0**			0 : 1 *0 : 1*		0 : 1 *0 : 1*	
68	3	0 : 6 : 3 *0 : 7 : 2* **0 8.33 0**			0 : 6 : 3 *0 : 7 : 2* **0 8.33 0**			0 : 9 *0.50 : 8.50*		0 : 9 *0.50 : 8.50*	
A324	5	2 : 7 : 1 *3 : 7 : 0* **38 15.29 0**			1 : 8 : 1 *0 : 8 : 2* **40 17.88 0**			2 : 8 *2.50 : 7.50*		1 : 9 *1 : 9*	
A271	7	1 : 21 : 7 *0 : 22.75 : 6.25* **31 13.75 0**			0 : 22 : 7 *0 : 23 : 6* **0 15.05 0**			1 : 28 *7.25 : 21.75*		0 : 29 *5.75 : 23.25*	
1915–1916											
4128	5	0 : 9 : 3 *0 : 7.50 : 4.50* **0 13.33 0**			0 : 9 : 3 *0 : 7.50 : 4.50* **0 13.33 0**			0 : 12 *1 : 11*		0 : 12 *0.50 : 11.50*	
619	1	2 : 0 : 0 *2 : 0 : 0* **39.50 0 0**			1 : 1 : 0 *1 : 1 : 0* **42 37 0**			2 : 0 *1 : 1*		1 : 1 *1 : 1*	

A272	1	1 : 0 : 0	1 : 0 : 0	1 : 0	1 : 0	3533	4	4 : 7 : 2	3 : 8 : 2	4 : 9	3 : 10
		1 : 0 : 0	*1 : 0 : 0*	*1 : 0*	*1 : 0*			*5.75 : 6.50 : 1.75*	*4 : 9 : 0*	*3.63 : 9.37*	*3.25 : 9.15*
		46 0 0	**46 0 0**					**54 17.14 0**	**43 19.12 0**		
A293	2	2 : 3 : 0	0 : 5 : 0	2 : 3	0 : 5	2914	3	4 : 4 : 2	0 : 8 : 2	4 : 6	0 : 10
		3 : 2 : 0	*0 : 5 : 0*	*2.50 : 2.50*	*0 : 5*			*3.87 : 5 : 1.13*	*0 : 7.75 : 2.25*	*6 : 4*	*1 : 9*
		35 21.66 0	**0 27 0**					**34.75 15.25 0**	**0 25 0**		
A271	4	2 : 11 : 5	1 : 12 : 5	2 : 16	1 : 17	2313	5	18 : 13 : 3	10 : 21 : 3	18 : 16	10 : 24
		4.75 : 9 : 4.25	*0 : 13.75 .75 : 4.25*	*4.25 : 13.75*	*4.25 : 13.75*			*20.75 : 13.25 : 0*	*10.25 : 17.50 : 6.25*	*18.50 : 15.50*	*11.50 : 32.50*
		36.50 16.36 0	**41 17.66 0**					**43.72 15.46 0**	**49.70 23.38 0**		
4167	9	15 : 26 : 12	9 : 32 : 12	15 : 38	9 : 44	3617	12	32 : 9 : 1	27 : 14 : 1	32 : 10	27 : 15
		14.75 : 26 5C : 11.75	*13.25 : 26.50 : 13.25*	*18.50 : 37.50*	*11.50 : 41.50*			*32.25 : 9.75 : 0*	*26.50 : 15.50 : 0*	*31.50 : 10.50*	*28.75 : 13.25*
		41.73 18.38 0	**44.88 21.87 0**					**50.69 17.44 0**	**53.92 23.07 0**		
A323	1	0 : 0 : 1	0 : 0 : 1	0 : 1	0 : 1	4265	3	1 : 7 : 1	0 : 8 : 1	1 : 8	0 : 9
		1 : 0 : 0	*0.50 : 1 : 0.50*	*0.50 : 0.50*	*0.50 : 0.50*			*3.50 : 5.50 : 0*	*0 : 7.50 : 1.50*	*2.13 : 6.87*	*1.25 : 7.75*
		0 0 0	**0 0 0**					**34 16.57 0**	**0 18.75 0**		
228	2	1 : 6 : 1	1 : 6 : 1	1 : 7	1 : 7	A143	4	2 : 8 : 6	0 : 10 : 6	2 : 14	0 : 16
		2 : 4 : 2	*2.75 : 4 : 1.25*	*2 : 6*	*2 : 6*			*2.50 : 9.25 : 4.25*	*1.25 : 8 : 6.75*	*3 : 13*	*1 : 15*
		66 18.50 0	**66 18.50 0**					**34.5 9.37 0**	**0 14.40 0**		
4260	3	8 : 2 : 0	5 : 5 : 0	8 : 2	5 : 5	68	5	2 : 5 : 4	2 : 5 : 4	2 : 9	2 : 9
		8 : 2 : 0	*5 : 5 : 0*	*7.50 : 2.50*	*5.68 : 5.32*			*4.13 : 5.50 : 1.37*	*4 : 5.50 : 1.50*	*3 : 7*	*2 : 9*
		43 14.50 0	**50 24.60 0**					**53 9.40 0**	**53 9.40 0**		
A269	6	2 : 10 : 1	1 : 11 : 1	2 : 11	1 : 12	A294 (Cornish)	2	0 : 0 : 4	0 : 0 : 4	0 : 4	0 : 4
		5 : 8 : 0	*1.50 : 11.50 : 0*	*3.13 : 9.87*	*0.75 : 12.25*			*0 : 0 : 4*	*0 : 0 : 4*	*0 : 4*	*0 : 4*
		39.5 14.70 0	**47 16.27 0**					**0 0 0**	**0 0 0**		
A274	8	20 : 14 : 0	14 : 20 : 0	20 : 14	14 : 20						
		24.50 : 9.50 : 0	*17 : 17 : 0*	*20.50 : 13.50*	*18 : 16*						
		45.20 19.92 0	**49.86 24.20 0**								

TABLE 5—*Continued*

Father's band number	Number of mothers	Pearl's theory: Division point in eggs 30: 30 and over	Pearl's theory: Division point in eggs 30: Under 30	Pearl's theory: Division point in eggs 30: Zero	Pearl's theory: Division point in eggs 40: 40 and over	Pearl's theory: Division point in eggs 40: Under 40	Pearl's theory: Division point in eggs 40: Zero	Alternative theory: Division point in eggs 30: 30 and over	Alternative theory: Division point in eggs 30: Under 30	Alternative theory: Division point in eggs 40: 40 and over	Alternative theory: Division point in eggs 40: Under 40
1916–1917											
6373	4	12	5	0	9	8	0	12 : 5		9 : 8	
		11.25	*5.75*	*0*	*10*	*7*	*0*	*12.50*	*4.50*	*8.75*	*8.25*
		43.42	**19.20**	**0**	**47.50**	**26.33**	**0**				
5240	1	6	0	0	5	1	0	6 : 0		5 : 1	
		6	*0*	*0*	*6*	*0*	*0*	*6*	*0*	*6*	*0*
		84.17	**0**	**0**	**93.50**	**38**	**0**				
4786	2	7	3	0	7	3	0	7 : 3		7 : 3	
		7	*3*	*0*	*7*	*3*	*0*	*5.50*	*4.50*	*8*	*2*
		69.86	**10**	**0**	**69.86**	**10**	**0**				
5077	3	9	2	0	7	4	0	9 : 2		7 : 4	
		9.25	*1.75*	*0*	*7.50*	*3.50*	*0*	*7.87*	*3.13*	*7*	*4*
		52.44	**15.50**	**0**	**57.57**	**25**	**0**				
6265	3	14	1	0	12	3	0	14 : 1		12 : 3	
		15	*0*	*0*	*13*	*2*	*0*	*15*	*0*	*13*	*2*
		58.54	**18**	**0**	**62.36**	**31**	**0**				
274	3	6	1	0	4	3	0	6 : 1		4 : 3	
		6.25	*0.75*	*0*	*3.50*	*3.50*	*0*	*6.25*	*0.75*	*4.13*	*2.87*
		41.28	**27**	**0**	**48.50**	**31.66**	**0**				
3617	5	30	5	0	24	11	0	30 : 5		24 : 11	
		31.75	*3.25*	*0*	*21*	*14*	*0*	*31.75*	*3.25*	*28*	*7*
		57.33	**20**	**0**	**62.71**	**28.63**	**0**				
5584	3	3	12	13	3	12	13	3 : 25		3 : 25	
		7	*14*	*7*	*5.50*	*14*	*8.50*	*7*	*21*	*5.50*	*22.50*
		46.33	**8.75**	**0**	**46.33**	**8.75**	**0**				
6781	4	23	9	1	19	13	1	23 : 10		19 : 14	
		24.50	*8.50*	*0*	*19.50*	*13.50*	*0*	*23.56*	*9.44*	*20.13*	*12.87*
		53.13	**10.11**	**0**	**57.42**	**17.07**	**0**				
4378	4	7	14	8	5	16	8	7 : 22		5 : 24	
		8.87	*14.50*	*5.63*	*7.25*	*14.50*	*7.25*	*9.62*	*19.38*	*4.75*	*24.25*
		45.14	**12.50**	**0**	**49.60**	**15.19**	**0**				
4723	6	47	18	2	36	29	2	47 : 20		36 : 31	
		50.75	*16.25*	*0*	*39.50*	*27.50*	*0*	*47.95*	*19.25*	*38.13*	*28.87*
		52.68	**17.11**	**0**	**38.30**	**23.62**	**0**				
5477	2	15	0	0	8	7	0	15 : 0		8 : 7	
		15	*0*	*0*	*7.50*	*7.50*	*0*	*15*	*0*	*7.50*	*7.50*
		41.20	**0**	**0**	**46.75**	**34.86**	**0**				
4354	4	29	6	0	27	8	0	29 : 6		27 : 8	
		31	*4*	*0*	*28.25*	*6.75*	*0*	*30*	*5*	*27.50*	*7.50*
		61.14	**12**	**0**	**63.15**	**17.50**	**0**				
5776	1	8	0	0	8	0	0	8 : 0		8 : 0	
		8	*0*	*0*	*8*	*0*	*0*	*8*	*0*	*8*	*0*
		59.87	**0**	**0**	**59.87**	**0**	**0**				

5581	6	35 : 6 : 0 *35.50 : 5.50 : 0* **57.14 13.67 0**	28 : 13 : 0 *27.50 : 13.50 : 0* **63.27 25 0**	35 : 6 *36.75 : 4.25*	28 : 13 *27.50 : 13.50*	4882	1	9 : 8 : 2 *9.50 : 9.50 : 0* **7.13 : 9.50 : 2.37**	5 : 12 : 2 *9.50 : 9.50* **4.75 : 14.25**	9 : 10	5 : 14
4312	4	4 : 9 : 1 *6.50 : 7.50 : 0* **51.50 14.67 0**	4 : 9 : 1 *4.25 : 7 : 2.75* **51.50 14.67 0**	4 : 10 *4.25 : 9.75*	4 : 10 *3.75 : 10.25*	Cornish *	3	0 : 2 : 1 *0 : 1 : 2* **0 14 0**	0 : 2 : 1 *0 : 1 : 2* **0 14 0**	0 : 3 *0 : 3*	0 : 3 *0 : 3*

1917–1918

8027	8	60 : 7 : 1 *61.75 : 6.35 : 0* **58.86 22.28 0**	52 : 15 : 1 *55.50 : 12.50 : 0* **63.23 30.18 0**	60 : 8 *61.75 : 6.25*	52 : 16 *53.68 : 14.32*	8147	7	41 : 11 : 0 *42.50 : 9.75 : 0* **60.56 18.73 0**	36 : 16 : 0 *36 : 16 : 0* **64.39 23.19 0**	41 : 11 *41.56 : 10.44*	36 : 16 *36 : 16*
7745	3	7 : 3 : 0 *8.50 : 1.50 : 0* **63 20.66 0**	7 : 3 : 0 *7 : 3 : 0* **63 20.66 0**	7 : 3 *8.50 : 1.50*	7 : 3 *7 : 3*	5470	4	7 : 8 : 0 *7.50 : 7.50 : 0* **35.85 15.25 0**	2 : 13 : 0 *5 : 10 : 0* **42.50 22.92 0**	7 : 8 *6.50 : 8.50*	2 : 13 *3 : 12*
8761	4	20 : 5 : 0 *22.50 : 3.50 : 0* **63 85 9.60 0**	19 : 6 : 0 *19.50 : 5.50 : 0* **65.63 13 0**	20 : 5 *22.25 : 2.75*	19 : 6 *19.25 : 5.75*	7727	2	11 : 0 : 0 *11 : 0 : 0* **52.50 10 0**	7 : 4 : 0 *5.50 : 5.50 : 0* **62.28 35.50 0**	11 : 0 *11 : 0*	7 : 4 *8.25 : 2.75*
8528	6	44 : 10 : 0 *44.50 : 9.50 : 0* **65.91 11.60 0**	41 : 13 : 0 *42.25 : 11.75 : 0* **68.39 16.31 0**	44 : 10 *44.50 : 9.50*	41 : 13 *40.75 : 13.25*	6373	3	10 : 5 : 0 *11.25 : 3.75 : 0* **51.40 17.40 0**	6 : 9 : 0 *7.50 : 7.50 : 0* **60.66 26.33 0**	10 : 5 *8.50 : 6.50*	6 : 9 *7 : 8*
8097	5	37 : 1 : 0 *38 : 0 : 0* **60.02 6 0**	35 : 3 : 0 *35.25 : 2.75 : 0* **61.48 25 0**	37 : 1 *38 : 0*	35 : 3 *35.25 : 2.75*	9752	1	29 : 38 : 0 *33.50 : 33.50 : 0* **42.27 18.42 0**	13 : 54 : 0 *25.50 : 41.50 : 0* **53.08 22.89 0**	29 : 38 *33 : 34*	13 : 54 *18.63 : 48.37*
8422	3	8 : 0 : 0 *8 : 0 : 0* **60.25 0 0**	5 : 3 : 0 *4.50 : 3.50 : 0* **77.25 32 0**	8 : 0 *8 : 0*	5 : 3 *5.25 : 2.75*	3003 9191		See Table 6. See Table 8.			
9305	1	2 : 7 : 1 *3.75 : 5 : 1.25* **35 11.71 0**	0 : 9 : 1 *0 : 10 : 0* **0 18 0**	2 : 8 *2.50 : 7.50*	0 : 10 *2.50 : 7.50*						

TABLE 5—*Concluded*

FATHER'S BAND NUMBER	NUMBER OF MOTHERS	PEARL'S THEORY						ALTERNATIVE THEORY			
		Division point in eggs						Division point in eggs			
		50			60			50		60	
		50 and over	Under 50	Zero	60 and over	Under 60	Zero	50 and over	Under 50	60 and over	Under 60
1917–1918 (High Families)											
8027	8	45 : 22 : 1			26 : 41 : 1			44 : 24		26 : 42	
		45 : 23 : 0			*39.50 : 28.50 : 0*			*44.25 : 23.75*		*29 : 39*	
		67.07	**35.11**	**0**	**75.96**	**42.93**	**0**				
7745	3	6 : 4 : 0			3 : 7 : 0			6 : 4		3 : 7	
		7 : 3 : 0			*5 : 5 : 0*			*6.37 : 3.63*		*3.25 : 6.75*	
		66.33	**26.25**	**0**	**80**	**37.57**	**0**				
8761	4	16 : 9 : 0			13 : 12 : 0			16 : 9		13 : 12	
		17.50 : 7.50 : 0			*12.50 : 12.50 : 0*			*16.25 : 8.75*		*15.37 : 9.63*	
		69.50	**23.66**	**0**	**72.61**	**31.75**	**0**				
8528	5	34 : 20 : 0			26 : 28 : 0			34 : 20		26 : 28	
		31 : 23 : 0			*27 : 27 : 0*			*35 : 19*		*27 : 27*	
		73.41	**26**	**0**	**79.38**	**34**	**0**				
8097	5	27 : 11 : 0			15 : 23 : 0			27 : 11		15 : 23	
		28 : 10 : 0			*19 : 19 : 0*			*27 : 11*		*14.37 : 23.62*	
		66.18	**44**	**0**	**76.20**	**47.13**	**0**				
8147	6	27 : 14 : 0			19 : 22 : 0			27 : 14		19 : 22	
		27.50 : 13.50 : 0			*20.50 : 20.50 : 0*			*28 : 13*		*19.50 : 21.50*	
		68.41	**31.57**	**0**	**74.63**	**39.59**	**0**				

* It was necessary to use two males interchangeably in order to secure any progeny whatsoever.

as a 'somatic' high, mediocre, or zero producer, save in a very few instances, and these all clearly result from some peculiarity of management, such as birds hatched late in May with records of nearly 30 eggs. Such exceptions are apparent rather than real.

In one or two instances females, such as female no. 6067 mated to male no. 5584, table 3, have been encountered where the number of high producers does not meet expectation. In the case just cited, the progeny were obviously subnormal in vitality, but were kept because they came from a high-hatching line. We can readily understand that cases may arise where a bird is genetically high although its record is mediocre, but it is hard to see how a bird genetically mediocre should markedly transcend the division line between classes. Some records, especially records not far above the division line are to be expected, but since it is a universal experience with Mendelian ratios that individual families frequently deviate markedly from expected ratios, although the average fit closely, it has seemed wisest to take this way out of such a difficulty, rather than assume that the genetic constitution is not represented by the somatic record.

In compiling the tables, the genetic constitution of each male is first determined by the ratios in which his offspring occur. When this result is checked against the parents of the male in question, it may happen that a male of the class indicated by his progeny could not have arisen from such parents. It is possible, however, to adjust all such discrepancies where a division point of 30 eggs is used. Doubtless adjustments can be made in the case of the other division points, but it does not seem necessary to pursue the matter further. Inability to make such adjustments would indicate that neither theory has any basis in fact.

The same care has been taken in classifying the females. To do so, however, is not as simple as it seems, for one change often involves others, and a long chain of changes is often necessary in order to reduce all the data to a harmonious whole. We have gone over the data with this end in view, for both theories with the division point at 30 eggs. With the single exception

of male 3003, as given elsewhere, every case encountered falls into line. The data, therefore, are reduced to a harmonious whole—a fact that speaks strongly for the validity of both theories. It is also noteworthy, where families are large, that they fit the scheme with practically no difficulty. We do not feel entirely confident, however, that it would be possible to accomplish the same results with a really adequate series of data, in which the families are of sufficient size. On the other hand, it is certain that one could use either scheme as a guide in breeding only if one knew definitely the gametic constitution of the birds he started with. This knowledge can be obtained either by a series of breeding tests extending over several years or else one must have available a progeny from each mother of twenty or more daughters. It is impossible to start as we have done and make the progeny and parental tests agree except by constant shifting of birds among the various gametic classes.

One family, sired by male no. 8027, when a division point at 60 eggs is used, fails to show a good agreement between observed and theoretical ratios on Pearl's theory, due to a deficiency in the expected number of birds laying over 60 eggs among the daughters of high producers. Such a deficiency is explicable, in part at least, because of the ease with which a record can be depressed below the division point, through environmental or managerial factors, for 60 eggs is at or near the maximum production for birds beginning to lay December 1. Thus, it is easy to understand why a portion of the daughters do not reach 60 eggs.

The only real exception to the application of Pearl's theory to our Rhode Island Red data is the case of the family sired by male no. 3003. The detailed data of this mating are given in tables 6 and 7.

In some respects the production of the daughters of this male is similar to that of the daughters of the Cornish male described in another section. In 1915 male no. 3003 was mated to several poor-producing birds, primarily in order to secure a flock of non-broody Rhode Island Reds. As will be observed from the table, all his offspring are either mediocre or zero producers.

TABLE

Matings of male no. 3003, giving the ratios for the April and May progeny of each mother. The data for 1915 and 1917 are tabulated separately

MOTHER'S BAND NUMBER	KIND OF PRODUCER	PEARL'S THEORY						ALTERNATIVE THEORY			
		Division point in eggs						Division point in eggs			
		30 and over	Under 30	Zero	40 and over	Under 40	Zero	30 and over	Under 30	40 and over	Under 40
1915 to 1916											
2453	Zero	0	3	3	0	3	3	0	6	0	6
3180	Under 30	0	0	3	0	0	3	0	3	0	3
3578	Under 30	0	1	1	0	1	1	0	2	0	2
A377	Under 30	0	0	2	0	0	2	0	2	0	2
Actual totals.....		0	4	9	0	4	9	0	13	0	13
Expected totals...		?			?			*3*	*10*	*2.50*	*10.50*
Average egg production.....		0	10.25	0	0	10.25	0				

MOTHER'S BAND NUMBER	KIND OF PRODUCER	PEARL'S THEORY						ALTERNATIVE THEORY			
		Division point in eggs						Division point in eggs			
		30 and over	Under 30	Zero	40 and over	Under 40	Zero	30 and over	Under 30	40 and over	Under 40
1917 to 1918											
2453	Zero	1	2	1	1	2	1	1	3	1	3
3180	Under 30	0	1	0	0	1	0	0	1	0	1
6267	Under 30	0	1	0	0	1	0	0	1	0	1
6982	Under 30	5	5	1	5	5	1	5	6	5	6
5832	Under 30	6	1	0	4	3	0	6	1	4	3
Totals.....		12	10	2	10	12	2	12	12	10	14
Expected totals..		?			?			*14.50*	*9.50*	*10*	*14*
Average egg production.....		46.08	14.80	0	47.60	18.75	0				

TABLE 7

Portion of family sheet of male no. 3003, giving pertinent data for each mother and daughter

1915–1916						1917–1918					
Mother's band number	Daughter's band number	Date hatched	Age at first egg	Date of first egg	Eggs to Mar. 1	Mother's band number	Daughter's band number	Date hatched	Age at first egg	Date of first egg	Eggs to Mar. 1
A332		*1912*		*Dec. 6*	*44*	*6267*		*May 2 1915*	*295*	*Feb. 21*	*6*
	4658	Mar. 14	326	Feb. 3	17		B1023	Apr. 15	221	Nov.22	15
2453		*Apr. 5 1914*	*339*	*Mar. 9*	*0*		B1881	May 6			Laid on floor
	5533	Apr. 11	339	Mar. 15	0						
	6540	May 16	307	Mar. 18	0	*6982*		*Apr. 27*	*235*	*Dec. 18*	*23*
	6773	May 23	267	Feb. 14	10		B 749	Apr. 8	227	Nov. 21	45
	6775	May 23	256	Feb. 3	7		B1035	Apr. 15	312	Feb. 21	1
	6776	May 23	288	Mar. 6	0		B1036	Apr. 15	314	Feb. 21	4
	6777	May 23	251	June 29	18		B1037	Apr. 15	223	Nov. 24	41
	7087	June 20	266	Mar. 13	0		B1038	Apr. 15	229	Nov. 30	49
							B1209	Apr. 22	274	Jan. 21	2
3180		*May 10 1914*	*282*	*Feb. 16*	*8*		B1210	Apr. 22	215	Dec. 18	25
							B1713	Apr. 29	264	Jan. 18	26
	5075	Mar. 28	356	Mar. 18	0		B1714	Apr. 29	325	Mar. 20	0
	5218	Apr. 4	358	Mar. 27	0		B1885	May 6	202	Nov. 24	40
	5469	Apr. 11	333	Mar. 9	0		B1886	May 6.	218	Dec. 10	41
	6821	May 30	397	July 2	0	*5832*		*Apr. 25 1915*	*261*	*Jan. 11*	*28*
3578		*May 24 1914*	*279*	*Feb. 20*	*2*		B 347	Mar. 25	227	Nov. 7	53
							B 348	Mar. 25	190	Oct. 1	52
	5403	Apr. 11	*		0		B 476	Apr. 1	228	Nov. 15	38
	6267	May 2	295	Feb. 21	6		B1020	Apr. 15	234	Dec. 5	50
							B1239	Apr. 22	276	Jan. 23	23
A377		*1912*	——	*Feb. 18*	*3*		B1562	Apr. 29	212	Nov. 27	55
	6651	May 23	310	Mar. 28	0		B1907	May 6	200	Nov. 22	45
	6652	May 2	*		0		B1909	May 6	211	Dec. 3	65
							B1910	May 6	207	Nov. 29	39
						3180		*May 10 1914*	*282*	*Feb. 16*	*8*
							B2474	May 20	256	Jan. 31	12
						2453		*Apr. 5 1914*	*339*	*Mar. 9*	*0*
							B1211	Apr. 22	215	Nov. 23	45
							B1785	May 6	259	Jan. 20	23
							B2385	May 20	231	Feb. 6	17
							B2466	June 3	285	Mar. 15	0

*Never laid.

In 1917 he was bred again for the same purpose as in 1915, but this time was mated to other birds also. The results for 1917 are in striking contrast to those of 1915, although the records of the new mates are only slightly better than the old. However, if the two years are combined, the actual ratio is 12 high: 14 mediocre: 11 zero, which may represent a theoretical ratio of 13.75 high: 18.50 mediocre: 4.75 zero.

Cornish male by Rhode Island Red female cross

The detailed data of this cross are given in table 8. The results of this experiment are of prime importance for theories of the inheritance of winter egg production, because it demonstrates, in the Rhode Island Red breed at least, that high production descends from mother to daughter. It is important to realize that the Cornish stock used in our experiments was obtained from the same source as Pearl's. Further, our data on the Cornish are in good agreement with the numerical results obtained by him (Pearl, '12). It is likewise of importance to note that the Cornish females bred to the same male as the Rhode Island Red females give a strikingly different result. If we attempt to reduce these data to the form of Pearl's theory, we at once become involved in difficulties. In the first place, no high producers at all are to be expected, yet we find that twenty-eight of the thirty-one individuals are high producers.[4]

While the average winter egg production (49.2 eggs) of the cross-bred pullets is not equal to that of their mothers (71.6 eggs), it is nearly equal to that (52.5 eggs) of the mother's families, i.e., the mothers and their sisters. The average production of the mother's families seems a fairer basis for comparison, as the mothers were a group of individuals with production much above the average, and regression would certainly be expected. The average hatching date of the five mothers is April 16; of their families, May 2; and of the cross breeds, April 19. The differences in time of hatching may

[4] Unless otherwise indicated, a high producer is a bird that lays 30 or more eggs before March 1 of her pullet year.

TABLE 8

Distribution of progeny of Cornish male no. 9191. The progeny of each mother is given separately

MOTHER'S BAND NUMBER	KIND OF PRODUCER	PEARL'S THEORY												ALTERNATIVE THEORY					
		Division point in eggs												Division point in eggs					
		30. Actual ratio			30. Expected ratio			40. Actual ratio			40. Expected ratio			30. Actual ratio	30. Expected ratio		40. Actual ratio	40. Expected ratio	
		30 and over	Under 30	Zero	30 and over	Under 30	Zero	40 and over	Under 40	Zero	40 and over	Under 40	Zero	30 and over : Under 30	30 and over	Under 30	40 and over : Under 40	40 and over	Under 40
Rhode Island Reds																			
8092	Over 40	6	1	0	*5.25*	*1.75*	*0*	4	3	0	*3.50*	*3.50*	*0*	6 : 1	*5.25*	*1.75*	*4* : 3	*3.50*	*3.50*
8400	Over 40	8	0	0	*8*	*0*	*0*	7	1	0	*8*	*0*	*0*	8 : 0	*8*	*0*	7 : 1	*8*	*0*
7972	Over 40	5	1	0	*4.50*	*1.50*	*0*	4	2	0	*3*	*3*	*0*	5 : 1	*4.50*	*1.50*	4 : 2	*3*	*3*
8393	Over 40	3	1	0	*3*	*1*	*0*	2	2	0	*2*	*2*	*0*	3 : 1	*3*	*1*	2 : 2	*2*	*2*
8339	Over 40	6	0	0	*6*	*0*	*0*	6	0	0	*6*	*0*	*0*	6 : 0	*6*	*0*	6 : 0	*6*	*0*
Cornish																			
9398	Under 30	0	3	2	*2.50*	*2.50*	*0*	0	3	2	*0*	*5*	*0*	0 : 5	*0*	*5*	0 : 5	*0*	*5*
9083	Under 30	2	1	1	*2*	*2*	*0*	1	2	1	*2*	*2*	*0*	2 : 2	*2*	*2*	1 : 3	*1*	*3*
4273	Under 30	0	0	2	*1*	*1*	*0*	0	0	2	*0*	*2*	*0*	0 : 2	*0*	*2*	0 : 2	*0*	*2*
Totals		30	7	5	*32.25*	*9.75*	*0*	24	13	5	*24.50*	*17.50*	*0*	30 : 12	*28.75*	*13.25*	24 : 18	*24.50*	*18.50*
Average egg production		**51.39**	**14.14**	**0**				**55.71**	**23.15**	**0**									

introduce a slight error in the comparisons. The error cannot be estimated, because two lots of birds, hatched a week apart may differ by as much as 30 per cent in average egg production. However the regressions from the mothers' families' average is about the same proportionally as Pearl observed in the case of the reciprocal cross, viz., Barred Rock males by Cornish females.

Several cross breds laid more eggs than their own mothers. This happened in two out of the five families. with an average excess of best daughter over mother of $21\frac{1}{2}$ eggs against $15\frac{1}{3}$ eggs of mother over best daughter in the other three families.

If the Cornish are not restricted to the three gametic classes, viz., 5, 6, 9, as is done by Pearl, it becomes possible on Pearl's theory, to secure a set of ratios that fit the observed fairly well, if it be assumed that the zero producers are physiological zeros. This assumption is plausible, because most of the Cornish are late maturing. Without this assumption it is impossible to find theoretical ratios in Pearl's theory that fit the observed ratios. Nevertheless, it will be observed from table 8 that considerable violence is done to the observed ratios in the case of one Cornish female, when an observed ratio of zero high to three mediocre to two zero producers is referred to a theoretical ratio of $2\frac{1}{2}$ high, $2\frac{1}{2}$ mediocre, and *no* zero producers! It is also necessary in other years to make similar changes in fitting observed to theoretical ratios, on the assumption that male no. 9191 belongs to class III.

There is still another difficulty to be explained on Pearl's theory, and that is the occurrence of a single Cornish female that is unquestionably a high producer. It is difficult to believe that she is a mutation because she is the daughter of a bird that though late hatched and somewhat slow about maturing, laid 27 eggs in a single laying period before March 1, i.e., has the characteristics of a late maturing high-producing female.

On the alternative theory, no difficulties are encountered in fitting observed to theoretical ratios.

Some of Pearl's observed results are in agreement with the results of our cross, particularly the progeny of Cornish male no. 578 (Pearl, '12, p. 373). Here a number of high-producing

birds appear where none are expected. Moreover, Pearl's data shows that 15.5 per cent of the F_1 offspring from Cornish males by Barred Plymouth Rock females lay more than 30 eggs, the average excess production being 15.7 eggs or more than 50 per cent. Now, the average winter production of these over-30 birds is almost exactly the same (viz., 45.7 eggs) as that (viz., 46.2 eggs) of the over-30 birds produced by Barred Plymouth Rock males on Cornish females. It should be observed further that in most cases the mean winter egg production of the over-30 group, in cases involving the use either of F_1 or pure Barred Plymouth Rock males is also low, ranging from 35 to 57.2 eggs, the average being 45.9. While it is not feasible to pursue the analysis further, because of the form in which the data is presented, yet in view of the facts presented above, it is not hard to believe that the results of these crossing experiments do not necessarily involve sex linkage.

THE BEARING OF MODIFICATIONS IN MANAGEMENT ON THE RESULTS

When these experiments were started, it was with the intention that the methods of management (i.e., the environment) to the smallest detail should remain constant throughout the work. Unfortunately, however, it soon developed that something was radically wrong with the method of rearing the chicks, which made changes here imperative. Later, results of the experiments themselves made certain changes in time of housing the pullets necessary. The various changes made are given in appendix 1.

Of the various changes there is only one, viz., a change in method of rearing the chicks, that appears capable of affecting the results to an appreciable degree, though it does so in two ways. First, this change now gives us with certainty normal adults to place in the laying houses. Second, it makes possible the maintenance of the laying flocks in a state of freedom from infectious disease, particularly roup. Roup, although very capricious in making its appearance, is favored by certain environmental conditions, especially the weather. Moreover, the

birds react variously. Some are apparently immune, others take the disease in a mild form and without apparent detriment to egg production, while others take it with various degrees of severity (our Cornish, for example, are particularly susceptible). Such individuals may cease laying entirely for a time or lay spasmodically. The presence of roup, therefore, complicates matters greatly.

The degree to which these changes in management have affected our records is a matter of surmise only. We have no precise data on this point. The evidence from the records themselves indicates that it may be of small moment as attested by the presence of low records in clean years. Nevertheless, the presence of these changes in management introduces an element of doubt, especially because of the variability in susceptibility of individuals and families to roup.

Size of families

There is a difficulty,[5] common to Pearl's data and my own, that renders it impossible at present to decide between the two theories and their several modifications and which renders it somewhat doubtful that either scheme has any foundation in fact. This difficulty lies in the fact that the adult female offspring of each pair are so few in number that it is almost always possible to refer any observed ratio to some theoretical ratio that will bring each mother into line with the rest of her group. Thus an observed 2:2 ratio will fit any of the following theoretical ratios, viz., 2:2, 1:3, 3:1, 3:5, or 9:7. Since the progeny of a single male in numerous instances is fairly large, it may be urged, by the law of errors, that the agreement between observed and theoretical ratios is adequate proof that the scheme correctly represents the actual mode of inheritance of winter egg production. This would be true only under certain conditions, which are: First, there must be no bias. Second, the sample of females mated must be a representative sample of the population.

[5] This difficulty is not confined to egg-production statistics, but unfortunately is encountered in much Mendelian work, particularly with mankind.

Third, the sample must be sufficiently large to include a proper proportion of all kinds of females. Fourth, the various females must produce approximately equal numbers of progeny. Not one of these points is fulfilled. The first, because it is evident that a bias exists which is the endeavor on the part of the interpreter of the data to secure a good fit between observed and theoretical ratios. No method exists by which this bias can be overcome. The second point is not met because the females are selected samples. The third, because the samples are comparatively small. The fourth, because some females produce many more progeny than others. It must also be remembered that the application of ratios is made to the progeny of each mother separately.

The importance of size of family is not merely an academic consideration, but very real as anyone who has worked with Mendelian ratios will readily appreciate. Even when dealing with the simple monohybrid ratio of a morphological character the progeny of a single pair often deviates widely from the expected ratio even when fairly large numbers are secured. The average number of adult offspring per mother in Pearl's experiments with pure Barred Plymouth Rocks is only 2.85, while in my experiments it ranges from 2.5 in 1913 to 6.97 in 1916. The largest family (i.e., offspring of one mother) of pullets I secured, hatched in April and May, is 19. One can only guess at the size of Pearl's largest family, but it cannot be much greater, for with the sexes evenly divided and with maximum production and perfect hatchability, the maximum number of April- and May-hatched pullets that can be expected in any one year from a single mother can hardly exceed 25. If 10 pullets per mother are available and if their records appear in the ratios of 4:6, 5:5, 3:7, or even 2:8, they would not be improbable deviations from 1:3, 3:5, or 1:1 ratios, for three birds moved from one side to the other in the extreme case of the 2:8 ratio changes it to equality. Thus, if one encounters a series of ratios which corresponds in general to that of a particular male mated with females of several types, but among which one female occurs that gives a ratio of 2:8, when equality is required, the

bounds of probability are not exceeded in considering the 2:8 ratio a chance deviation from equality. The same sort of reasoning applies if we recognize three sorts of winter layers instead of two.

In order to secure families (i.e., progeny of a single pair) of sufficient size, hatched at the proper time, and fulfilling the other conditions necessary, it is necessary to repeat identical matings of a critical nature through a period of years. To do this would require huge physical facilities. It will therefore be difficult to secure suitable data.

There is another consideration that affects the application of small observed ratios to theoretical ratios. Any individual is placed in one of two (three, respectively) classes. It is obvious, then, that ratios such as the following 1:0, 0:1, 1:1, 1:2, 2:1, 1:3, 3:1, 2:3, 3:2 and so on will occur as a matter of chance.[6] It is obvious, furthermore, that only when it is possible to obtain a really adequate number of female progeny, not less than 20 from each pair, will it become possible to determine definitely whether or not either theory has any basis in fact. As Castle ('15, '16) has maintained, we may be dealing with a character that is purely continuous in its variation.

The ease with which any observed set of ratios in small families can be made to fit at least one theoretical ratio is emphasized by the changes in management as given in an earlier paragraph. Thus, no difficulty is experienced in securing a close fit between expected and theoretical ratios in 1914, although the egg production of the progeny of the males used that year was entirely different from that of the same males in other years. Of course it is necessary to assign the males to different gametic classes in the different years. Under such circumstances, it is obvious that Mendelian ratios may not express the true mode of inheritance of fecundity.

[6] The series for three classes are 1:0:0, 0:1:0, 0:0:1, 1:1:0, 0:1:1, 1:0:1, 1:1:1, 2;1:0, 2:0:1, 0:2:1, and so on.

RATIOS AMONG THE PARENTS NEEDED TO GIVE THE OBSERVED MEAN WINTER EGG PRODUCTION OF AMERICAN BREEDS WITH RANDOM MATINGS

The mean winter egg production of several American breeds of poultry is about 36 eggs as has been pointed out by Pearl ('15b). This average results from random matings. To secure this average requires that the ratios in which the various classes of females appear shall be 12 high, 8 mediocre, and 1 zero, if the average winter production for the high group is about 55 eggs. The high-producing Barred Plymouth Rocks studied by Pearl ('12) averaged 53.08 eggs, the mediocre 15.58, and occur in the observed ratio of 360.5 high to 252.5 mediocre to 30 zero, which is close to that expected. If 36 eggs or thereabouts is the general winter average of American breeds that are properly managed, it follows that the three classes of females should occur in the proportions given above. To produce females in this ratio with random matings requires that the various classes of males also appear in certain definite ratios. We have determined one set of ratios (percentage), viz., 14.7 : 15.5 : 25.0 : 3.9 : 3.3 : 5.3 : 11.0 : 19.2 : 0.2, that with females in the ratio of 12 high: 8 mediocre: 1 zero (percentage ratios, 57.1 high, 38.1 mediocre, 4.8 zero) reproduces very nearly the initial ratio among the female offspring. It does not, however, exactly reproduce itself among the males. Whether or not it is theoretically possible to secure a ratio among the males that with random matings will yield females in the proper proportions and reproduce both itself and the proper female ratio in each generation we will leave to those who have the necessary taste and attainments in mathematics. It is evident, however, that such theoretical ratios must exist if either theory has any basis in fact.

CRITICAL VALUE OF MALES OF CLASSES I, II, AND V FOR PEARL'S THEORY

There are three classes of males, viz., *I*, *II* and *V*, that have a critical value in determining the validity of Pearl's theory, because the ratios in which their daughters appear is the same whatever the mother may be. Thus, class *I* males throw all

high females, class *II* males throw half high and half mediocre, while class *V* throw only mediocre. Neither Pearl nor myself have many satisfactory data on such males. Pearl notes but one class *I* male, and this one mated to 10 females had an average progeny per mother of 1.8 daughters. There were four class *II* males. They have a total progeny of 35 daughters and an average of 2.7 daughters per mother. These families are wholly unsatisfactory. No class *V* male appeared.

Several Rhode Island Red males have appeared that throw all or nearly all high producers, but not all kinds of females were mated to them. Two class *II* males are recorded. The number of progeny of one is small while one of the mates of the other gave a ratio of 11 high: 4 mediocre: 0 zero against an expected ratio of $7\frac{1}{2}$ high: $7\frac{1}{2}$ mediocre: 0 zero. No class *V* male appeared.

It is somewhat difficult to ascertain even roughly the proportion in which the males of the various classes should be encountered, though it is clear that class *V* males are to be expected only rarely. If we assume that the proportions given in a preceding paragraph are approximately correct, then classes *I* and *II* should appear at least as frequently as *VII* and at least half as frequently as *III*. They do not do so, however (table 9).

It appears probable that the validity or non-validity of Pearl's theory could be demonstrated beyond doubt by first obtaining males supposed to belong to classes *I*, *II*, and V and then breed-

TABLE 9

Observed numbers of males in each class as determined by Pearl's theory

FLOCK	CLASS OF MALES								
	I	II	III	IV	V	VI	VII	VIII	IX
Barred Plymouth Rocks (Pearl 1912)	1	4	2	4	0	0	11	1	0
Rhode Island Reds, Mass. Agricultural Experiment Station	7*	2	18	5	0	1	13	5	1

* This includes males inadequately tested because mated to high producing females only.

ing them extensively to the several sorts of females, to see whether or not large families could be obtained having the required ratios.

SUMMARY OF CRITICISMS OF BOTH THEORIES

In several places we have indicated difficulties encountered in applying Pearl's theory of the inheritance of egg production to our data and which affect the validity of that theory. Sufficient data, however, are not at hand to wholly disprove the theory, though the Cornish male by Rhode Island Red female cross demonstrates that the theory is not of universal applicability.

These difficulties mentioned, however, render necessary a state of suspended judgment even in rspect to the applicability of Pearl's theory to his own data. These difficulties may be summarized here:

1. The extremely small size of the individual families and the various consequences that flow therefrom.
2. Results of the Cornish, Rhode Island Red cross.
3. Occurrence of high producers where none are expected.
4. The too great ease with which abnormal production may be made to fit theoretical ratios.
5. The lack of class *I*, *II*, and V males mated to all sorts of females with families of adequate size.
6. The facility with which the several division points between high and mediocre producers may be employed.

Difficulties 1, 4, and 6 apply also to the alternative theory.

CONCLUSIONS

The conclusions to be drawn may be stated as follows:

1. The mode of inheritance of winter egg production remains to be determined.
2. The validity of Pearl's theory can be settled only by breeding operations conducted on a large scale, with disease and the necessity of practical considerations eliminated. It should be borne in mind, however, that as Pearl's mediocre producers are birds that lay at a slow rate, irregularly and spasmodically,

while mine are mainly birds of late maturity, that the numerical results obtained may be wholly valid for his strain. Moreover, in the Barred Plymouth Rocks the winter cycle of production is a characteristic feature of nearly all records. It, therefore, remains entirely possible that Pearl's theory is fully applicable to his particular strain of Barred Plymouth Rocks, in which case it should be stated as a theory of the inheritance of rate of production during the winter cycle. Looked at from this angle, then, it is apparent that Pearl's theory may be a complete explanation of this phase of the inheritance of egg production.

Although our numerical data are similar to those of Pearl, we believe that the methods used in this paper are wholly inadequate for the solution of the problems of inheritance of egg production. On the contrary, the problems should be approached from an entirely different angle, namely, that of the inheritance of the several factors, whose combined action results in a given number of eggs for the winter period (compare Goodale, '18).

SUMMARY

1. The data furnished by the flocks of Rhode Island Reds at this station furnish ratios that agree with those expected on Pearl's theory of the inheritances of fecundity.

2. A division point at 40, 50, or even 60 eggs gives ratios that agree well with the observed ratios.

3. An alternative theory that recognizes two classes of winter egg production, viz., high and mediocre, and assumes two genes, both of which must be present in the female zygote in order to have high production, and which are inherited according to the Mendelian dihybrid scheme, will also account for the observed ratios.

4. Reasons are presented, showing that the applicability of these schemes may depend upon the small average number of offspring produced by each pair of parents. Methods by which the falsity or truth of these schemes may be established are given.

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APPENDIX 1

Changes in management

At the outset of these experiments, it was determined that methods of management should remain constant; but, unfortunately, serious difficulties in rearing the young stock appeared and radical changes were necessary. As later events demonstrated, the trouble was disease, and not methods of management in the narrow sense. We were much more fortunate in the methods selected for handling the adult stock and in the selection of our methods of incubation. It is the purpose of this section to describe briefly such changes as have been necessary in order that the reader may be able to understand their bearing on the results of the breeding work.

Adults. Feeding. Rations and methods of feeding have remained constant, except in the sort of green food fed. Whenever possible, green cabbage has been fed during the winter, but on a few occasions it has been necessary to substitute mangels. At other seasons any green feed available has been fed.

Housing. Large open-front houses have been used, except in 1913–14 when about half the pullets were placed in six small pens (accommodating twenty-five to thirty birds each) of a long open-front laying house. The large houses are of two types, but very similar. It has never been possible to observe any difference in production attributable to the differences in housing.

Numbers in flock. One type of the large house was built for 72 birds to a pen, the other for 100. The partitions are solid so that each pen is virtually a separate house. During recent years, in order to accommodate the birds, it has been necessary to place more than the theoretical number of birds in each pen, as high as a 70 per cent increase having been made in one instance. This theoretical overcrowding, if it has any effect at all, should result in decreased egg production, and therefore in a direction opposite the observed results of the experiments.

Time of placing birds in the laying houses. At first the pullets were placed in the laying houses late in October or in November, according to age. No eggs, however, were laid on the range.

As the pullets have matured earlier and earlier each year it has become imperative to get them into the laying houses much earlier, if possible before laying commences. The earliest hatched pullets now go in early in September, and the later hatched, by the first week in October. To make room for the pullets, the birds of the preceding generation are moved into outside roosting sheds sometime during the summer.

Chick rearing. In 1913 and 1914, the chicks were brooded in the long-pipe brooder house and grown on the College range. The mortality,[7] however, was so great that in 1915 resort to the small hovers was made. This season was devoted to the establishment of a satisfactory method of brooding, which, with one or two final adjustments, has been kept as uniform as possible since then.

Chick rations. During 1916 and 1917 the rations were constant. In 1915, they varied from flock to flock. Those employed in 1914 and 1913, though unlike each other and unlike any of the other years, were constant throughout each season. However, all these various rations were fully adequate to promote rapid growth and cannot have had any effect on subsequent egg production.

Disease. Adults. One of the most difficult problems to contend with in the management of poultry is the appearance of disease. It is a formidable difficulty in the way of securing consistent results year after year and is unquestionably the most important factor in preventing one from keeping poultry under uniform environmental conditions, even more important than the weather. During the course of these investigations, a method of eliminating infectious disease has been developed. The records for 1916 to 1917 and the winter of 1917 to 1918 are virtually free from the influence of infectious disease.

Chicks. *a.* White diarrhea. Although bacteriological examinations were made on dead chicks for this disease in 1913 and 1914 by the Department of Veterinary Science, it was not dis-

[7] This mortality was due mainly to white diarrhea, and not to the brooder house, as later event proved.

covered until 1915. Later, when the agglutination test was applied to all breeders, reactors belonging to the original flock were found. Thus most of the mortality during the first three weeks after hatching in 1913, 1914, and 1915 must have been due to this disease, as no difficulty is experienced at present in rearing chicks. During and since 1915, the experimental flocks have been free from this trouble.

b. Filth diseases. There is a group of diseases about which very little is known, but which we infer are spread by filth. It has been the common practice for years to clean and disinfect brooders or colony houses, place baby chicks therein, take pains to clean the brooders frequently while the chicks are growing, but no attention is paid to the ground over which the chicks run, which may be the adjoining hen yard, nor does the attendant make the slightest effort to avoid the transfer of filth from the adult birds, especially by means of his feet. If, however, adequate measures are taken to prevent such contagion, trouble from this source disappears. In addition to bacillary white diarrhea, trouble from this class of diseases was experienced in 1913 and 1914, and in that part of the flock of 1915 which was not isolated. In 1915, most of the chicks were reared under isolation. In 1916 and 1917, all the chicks were reared on clean ground by a special attendant.

APPENDIX 2

Remarks to practical poultrymen

Theories of egg production are of little interest to the practical man unless they can be turned into actual eggs. The poultryman has been told first one thing and then another about breeding for more eggs until the time is approaching when he will believe that it is all humbug. The situation is, indeed, confusing. This is because the various theories that have been and will be developed are necessarily attempts at reducing the available facts to order. They should be regarded as reports of progress and subject to revision. Theories, however, are extremely useful, because they stimulate further investigation

and add new facts and new points of view. Pearl's theory has been very valuable, because it has drawn attention to the importance of the male's influence on the egg production of his daughters. That influence is demonstrated, even if it is not all important as it seemed at first.

Fortunately, in spite of the confusion of theories, there is a solid basis of fact on which the poultryman may proceed who wishes to improve his egg production through breeding. Pearl and Dryden have shown that flock egg production is increased by suitable methods of selecting and testing the breeders.

We, too, find that selection gives results. The method of selection, moreover, is so simple that anyone can use it who is willing to use trap nests and keep the necessary records. The essentials of the method are these, assuming that the start is made with unpedigreed stock. First, the stock must be perfectly sound and vigorous. Second, select breeders that equal or exceed some definite number of eggs and mate them to the strongest males available. Third, put only strong (but not necessarily large) healthy pullets in the laying houses. Fourth, when the daughters' records are available, group them together by mothers. Fifth, pick out the best and second best families. Select the breeding males from these families, taking care to pick the strongest and most vigorous individuals. Mate these to the best pullets of the best families. Proceed in this way testing each year the results of each mating by the records made by the progeny.

One should not depend wholly on the numerical record in selecting the breeders. Look carefully to the age at which each pullet commences to lay. Note whether or not she lays continuously or takes vacations. Note whether she lays rapidly or slowly, or whether she lays late into the fall, and the number of times she becomes broody.

On the commercial plant it would probably be unwise to trapnest more than 200 birds annually. The best 10 or 15 per cent of these should be used to produce the next flock to be trapnested, and the remainder used to propagate the general flock.

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THE FATE OF HOMOZYGOUS YELLOW MICE

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TWO FIGURES

INTRODUCTION

The failure of breeders and investigators to obtain any homozygous yellow mice and also the observed smaller average litters born to yellow parents (Cuenot, '05; Castle and Little, '10) indicated the advisability of attacking this problem from the embryological side, a conclusion justified by the satisfactory outcome shown below. A preliminary report on the present research was made before the American Society of Zoologists in 1916, published as an abstract in the Anatomical Record of the following year. Since then Ibsen and Steigleder ('17) have published the results from a somewhat similar piece of work, but limited to embryos from females thirteen to nineteen days pregnant; the details of this paper will be considered in connection with those of the present investigation.

ANALYSIS OF THE PROBLEM

White mice, and probably other mammals (compare Meyer, '17), encounter two crises in their lives even before they are born, the first occurring at the time of implantation in the wall of the uterus, and the second at parturition. Loss of homozygous yellow offspring at parturition would, if it took place, have been observed by previous investigators, and their failure to record either still births or moribund young as regular occurrences after yellow matings is supported by the findings of the present writer. There remained, therefore, the crisis at the time of implantation as a likely key to the yellow-mouse problem.

The mouse ovum leaves the ovary with a supply of food material sufficient, with what serous fluid is available for absorption in the fallopian tube and uterine cavity, to maintain its metabolism during fertilization and subsequent cleavage, through the formation of a blastula. These various processes appear to involve very stable mechanisms, for although in mice of all colors a small number of eggs are eliminated immediately after ovulation through inability, or accidental failure, to unite with sperm cells, eggs which have been fertilized and yet are so pathological as to have had development brought to a standstill between the stages of fertilization and implantation are very rarely seen. Implantation, on the other hand, evidently involves a much less stable set of factors than does cleavage, since in white mice counts of thirty-four sets of cleaving ova and of blastulas average more than seven individuals to the set, while thirty-three litters of new-born young obtained during the same period from mice living under identical conditions and of the same stock averaged less than five. Further evidence for the above statement is the fact that the writer's stock has never yielded a litter of more than nine young, while one set of ten cleaving eggs, another of thirteen, and a set of eleven blastulas have been found, and, as will be again stated later, in mice other than yellow, degeneration of embryos after implantation is rare, and when it occurs is due in most, if not all instances, to infection.

Many unsolved problems remain in connection with implantation, but there exists now sufficient evidence to render certain the existence of two distinct sets of factors, one maternal, the other embryonic. The work of Fraenkel and Cohn ('10), Loeb ('08), Marshall and Jolly ('10), and others has shown that the corpora lutea are responsible for a stimulus which causes the uterine mucosa to proliferate, a necessary process antecedent to the implantation of the blastulas. Adler ('12) and the writer (Kirkham,'18) have also proved that when lactation is in progress the mammary glands exert some sort of inhibitory influence upon both the stimuli reaching the uterine mucosa from the corpora lutea and those coming from the blastulas. The stimulus from the mouse blastula causes first a further swelling

and proliferation of the cells of the uterine connective tissue, followed shortly by the dissolution of both these cells and those of the overlying epithelium, thus supplying food for the embryos and effecting a closer union between the embryonic and maternal tissues.

It is still unknown why in large sets of blastulas not all become implanted, but the writer has seen no evidence in white mice of morphological defects in any members of such sets, prior to implantation, and it more likely depends on either physiological deficiencies present in individual cases, on a time factor, or on these two factors acting together. All the blastulas of a set do not reach the uterus simultaneously, nor do they all implant at the same moment, and it is conceivable that the conditions are such that as the separate blastulas implant, the uterus offers an increasing resistance to those remaining free. Whatever the cause, it is at least certain that in normal mice, other than yellow, the blastulas that are unable to implant fail to arouse the specific response from the uterine wall, soon become moribund and are phagocytized.

Once implanted, mouse embryos of all colors appear well able to meet the demands of development up to the time of parturition, and the few instances of dead implanted embryos seen by the writer and those designated 'B' embryos by Ibsen and Steigleder ('17) are almost certainly due to pathogenic conditions in the maternal tissues. When such conditions are extreme, an entire litter may die in utero, while less critically adverse environments will cause the death of only the less resistant embryos, for before, as well as after, birth they are individualized organisms.

At the time of birth the second crisis occurs, and feeble mouse embryos of all colors which have succeeded in reaching this crucial stage in their lives succumb to the difficulties inherent in the transition from a parasitic to an independent existence.

OBSERVATIONS

The yellow-mouse material used in this investigation consists of complete series of sections of ovaries and fallopian tubes from healthy, non-suckling females representing each of the first three days of pregnancy, and like material, with the addition of entire uteri with all contained embryos for each day of pregnancy from the fourth to the twentieth.

There were also available four sets of preparations from sickly females. As a control there were full sets of similar material from healthy, non-suckling white mice, and serial sections of seventeen degenerating white-mouse embryos, at least sixteen of which came from sickly females; all of these white mice coming from lines which had been subjected to cross-breeding with those of other coat colors, and found to be without the factor for yellow. The details of the yellow-mouse material are set forth in table 1.

The time of ovulation, with respect to previous parturition, the time of fertilization, rate of cleavage, and time of implantation are the same for all mouse ova, whether whites, homozygous yellows, or heterozygous yellows, and the number of cleaving eggs, morulas, and blastulas found in individual yellow females falls within the same limits as found in white females, thus disposing as mathematically invalid, of any theory that the missing homozygous yellows are to be accounted for on the basis of the failure of eggs with the yellow factor to undergo maturation or fertilization. The two-cell stages examined all appear normal, but by the time the eggs have reached the morula stage in yellow mice certain ones show abnormalities, thus one of the morulae in set Y 40 has very indistinct cell boundaries. Abnormal blastulas can be distinguished easily by their shrunken appearance, the cells in such cases being small and crowded, and the blastodermic vesicle smaller then normal (fig. 1). Nevertheless, these abnormal individuals were alive at the time of preservation, and it is not until they are due to implant that they begin to plasmolyze and are overwhelmed by phagocytes.

TABLE 1

Sets of yellow-mouse embryos

AGE IN DAYS	SERIAL NUMBER	NORMAL EMBRYOS	DEGENERATE EMBRYOS	REMARKS
1	Y38	5	0	
2	Y18	9	0	
3	Y40	3	1	
3	Y42	6	1	Stillbirth in preceding litter
4	Y36	7	3	
5	Y39	7	0	Stillbirths in preceding litter
5	Y41	7	1	
5	Y44	8	1	
6	Y30	3	3	
6	Y45	6	3	
7	Y27	8	0	
8	Y19	4	3	
9	Y33	3	4	
10	Y13	5	3	Preceding litter eaten
10	Y28	5	1	
11	Y23	5	3	
12	Y20	4	3	
13	Y24	1	1	
14	Y22	2	4	
15	Y32	3	3	
16	Y21	5	0	Preceding litter eaten
16	Y47	5	1	
17	Y25	2	1	
17	Y43	3	2	
17	Y48	7	1	
18	Y26	7	0	
19	Y31	1	0	

The destruction of abnormal blastulas by phagocytes (fig. 2) is a very rapid process, similar to that described by Charlton ('17) for unfertilized mouse eggs, but in these cases probably a matter of only a few hours, for it does not start until the embryos reach the uterus, and by the time the normal ones are implanted, the abnormal ones are represented merely by masses of phagocytes and a few cell fragments. Whatever the exact nature of the influence that attracts the phagocytes, it is evidently active as soon as the abnormal blastulas reach the uterus, for several cases were observed where certain free blastulas were being

attacked before any other members of the sets had started to implant.

Swift as is the destruction of abnormal blastulas after they reach the uterus, it is nevertheless in yellow mice not so rapid as to prevent them from secreting the substance which causes the swelling and proliferation of the uterine mucosa, for this process takes place in a normal manner, the epithelium dis-

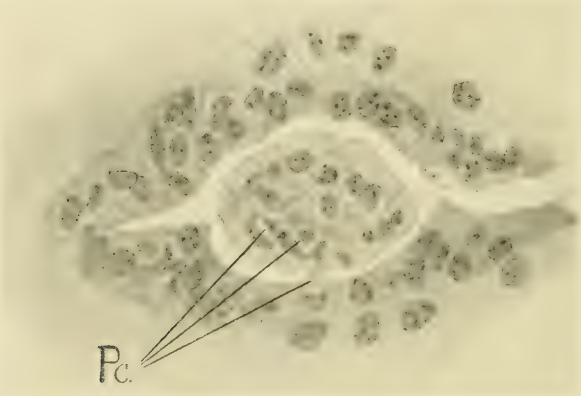

Fig. 1 Abnormal blastula from a yellow mouse, six days after ovulation (set Y 45). This set of embryos consists of five normal and three abnormal blastulas; two of the latter are illustrated in this and the following figures, and the third is similar to this one. The normal set mates of this specimen are in an early implantation stage, and while the uterine epithelium near this abnormal individual is still intact, the neighboring mucosa is much swollen. The section drawn passes through the middle of the blastula, but the cavity is small and so located with respect to the plane of sectioning that it is merely indicated by the lighter, central area. Three invading phagocytes are shown (*Pc.*), and several others appear in adjoining sections. × 600.

appears, the lumen of the uterus is closed, and all this goes on even though the blastula itself is being rapidly dissolved. This is in marked contrast to the fate, mentioned above, of abnormal blastulas in healthy non-yellow mice, which perish without effecting the uterine reaction. However, the same phenomenon of a normal, fully prepared implantation site with no trace of embryonic cells likewise occurs in about 50 per cent of the pregnant white females examined, which had either produced still-

births or had eaten the young at the time of the preceding parturition. An intensive study of such examples from both yellow and white mice has failed to reveal any differences between

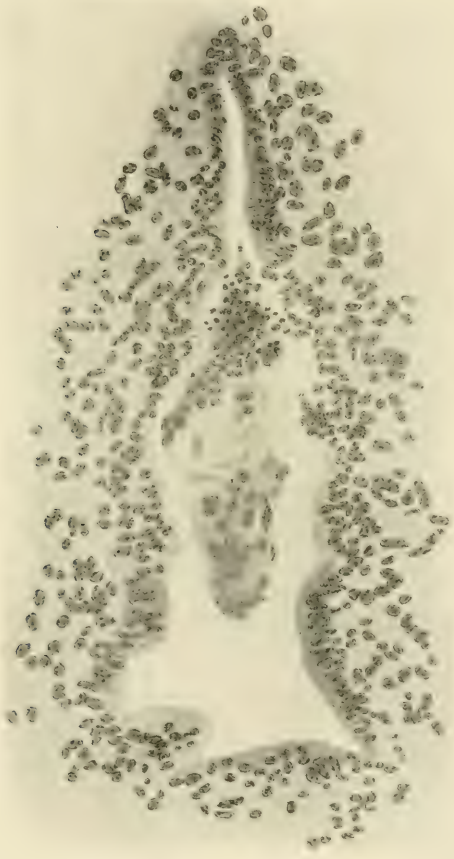

Fig. 2 Abnormal blastula from the same set as the one shown in figure 1. This individual developed further than the one illustrated above, being larger, as well as more normal in form, and progressed further toward implantation. Phagocytes have overwhelmed one pole, and all the cells of the blastula are undergoing cytolysis. × 240

them. In both kinds the uterine lumen remains closed until the fifteenth day of gestation, although the destroyed epithelium is regenerated a day or two before this. No 'wandering' cells

appear in these specimens, which offers some additional support to the statement of Asai ('14) that this type of cell is embryonic in origin.

The similarity in the histological details of the absorbed embryos in white and in yellow mice might be taken to prove the identity of the underlying causes in the two cases, but the associated facts tend to modify any such view. The degenerate embryos from white females were obtained, with possibly one exception, from animals which were more or less pathological, and the one possible exception is quite likely not such, as the preceding litter in that instance was removed at birth and otherwise might not have survived for long. Thus in white mice the absorbed embryos might all be accounted for on the basis of pathologic uterine environment which selectively disposed of the weaker members of the sets of blastulas, the set mates surviving. The same factor may be present in all yellow females, but in these animals, instead of the unfavorable environment being abnormal, we should have to assume, on account of the universality of the phenomenon, that it is actually a normal correlation with yellow coat color, an assumption further supported by the marked tendency in yellow mice of both sexes to fatness and sterility at a relatively early age. This matter will be subjected to further investigation through a projected study of the non-yellow offspring from yellow matings which, if the above assumption is correct, should be differentiated in general vitality from control animals, offspring from non-yellow parents.

Parental abnormality, however, cannot account for all the facts connected with the failure of homozygous yellow mice to be born, and we must further assume an inherent weakness, or lethal factor, in all the homozygous yellow embryos which invariably brings about their destruction during implantation, while their fellows in the same ovulation and environment, but endowed with factors associated with a different coat color, implant and develop normally.

The assumption is warranted that the degenerate embryos found regularly in pregnant yellow mice mated with males of the same coat color are the missing homozygous animals, for their

occurrence agrees numerically with the requirements of such a case, but a definite proof that such an assumption is valid must await future experimental inquiries. If it is possible at some future time to transplant the ovaries from a yellow female to a mouse of another coat color, it is conceivable that a subsequent mating with a yellow male might, under the assumed more favorable uterine conditions thus obtained, produce viable homozygous yellows.

The statistical evidence from this research is presented in table 2, where embryos less than three days old are omitted, owing to the failure of abnormalities to become evident before the morula stage.

TABLE 2

Showing percentage of degenerate embryos in yellow as compared to white mice

	YELLOW MICE	WHITE MICE
Total sets of embryos from healthy non-suckling females 3 to 20 days pregnant	21	26
Total normal embryos	94	189
Total degenerate embryos	39	2
Percentage of degenerate embryos	29+	1.0+

The data show clearly that the degenerate embryos occurring quite regularly in healthy yellow females mated with males of the same coat color must be considered as of a quite different nature from those occasionally found in white mice, for the latter are found almost only, if not invariably, in unhealthy females. The evidence thus strongly indicates that the former are the missing homozygous animals. The proportion of degenerate embryos in normal yellow females is, including additional material obtained since the publication of the preliminary report, 29+ per cent, which is but little higher than the Mendelian expectation of 25 per cent, and is quite within the limits of probability when the total amount of the material is relatively small.

CONCLUSION

Three points stand out in a survey of the results attained in this investigation.

First, all mouse embryos encounter a crisis at the time of implantation of the blastula in the wall of the uterus, and in unusually large sets of blastulas one or more appear always to perish at this time without producing any uterine reaction. In healthy mice other than yellows, however, those blastulas which induce a swelling of the mucosa uniformly complete their implantation, while the blastulas resulting from yellow matings almost always lose at least one of each set after the mucosa has reacted.

Second, apart from this loss of certain blastulas during implantation, the embryonic and early postnatal history of yellow mice is exactly the same as that of mice of other coat colors.

Third, the evidence that the blastulas lost in yellow females during implantation are the missing homozygous yellow mice consists on the one hand of the absence of any like phenomenon in healthy white mice, and on the other hand of the statistical correspondence of the percentage of embryos so lost with the Mendelian expectation of homozygous yellows.

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Resumido por el autor, Carl R. Moore.

Sobre las propiedades fisiológicas de las gonadas como reguladores de los caracteres somáticos y psíquicos.

I. La rata.

Un estudio de las modificaciones que siguen a la gonadectomía en las ratas jóvenes y el transplante ulterior de la gonada opuesta en cada animal (repetición de los experimentos de Steinach), demuestra que los efectos aparentes de tal experimento no son tan marcados como podría esperarse después de leer las comunicaciones de Steinach. Es muy difícil demostrar la presencia de cambios somáticos debidos en absoluto a la presencia de la glándula transplantada. Bajo el punto de vista psíquico los resultados son mas definidos. Los machos jóvenes han sido transformados aparentemente en hembras tan típicas que el instinto maternal de protejer y criar a los pequeños puede notarse inmediatamente. Las hembras jóvenes han sido también transformadas en machos hasta tal punto que se conducen psíquicamente como tales y los imitan, de una manera muy exacta, en el acto de la cópula. Los cortes histológicos de los ingertos demuestran que el ovario ha persistido aparentemente funcional, mientras que el testículo ha sufrido cambios marcados que conducen a la destrucción de los espermatocitos y espermatozoides.

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ON THE PHYSIOLOGICAL PROPERTIES OF THE GONADS AS CONTROLLERS OF SOMATIC AND PSYCHICAL CHARACTERISTICS

1. THE RAT

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FIVE FIGURES

In several papers during the past few years E. Steinach has described many changes occurring during the development of mammals, both of a somatic and of a psychical nature, which he affirms are due to a secretion of the sex gland.[1] He reports that an ovarian graft in a completely castrated young male rat or guinea-pig will so modify the subsequent development that the animal becomes somatically and psychically a female. These animals are proportionately lighter in weight, shorter in body length, hair smooth and fine, and pelvis smaller than that of males of the same age. Also these 'feminized males' react more like females than males (more docile, absence of male instincts toward female rats, reactions toward young characteristic of that of a mother). In guinea-pigs growth of the mammary glands and milk secretion was also reported. In the reverse experiment, i.e., if pieces of testicular tissue are grafted into completely spayed young females, the animals become masculinized as maturity is reached; they resemble males instead of females both somatically and psychically.

Steinach supposes that a secretion from the interstitial cells of the grafted testis and ovary in each case is the controlling factor since the secondary characteristics of the opposite sex do not appear unless the implanted gonad obtains vascular con-

[1] See Steinach, '10, '11, '12, '13.

nections and remains in a living condition after the transplantation. It is thought the modification is affected by a hormone produced by the interstitial cells, that its action is a chemical one, and that it may sensitize the nervous system to react in a new capacity.

These results have been criticised severely by many investigators, and to the writer's knowledge the observations have not been corroborated in other laboratories. In regard to criticisms the following annotation from Lancet (vol. 193, no. 18 of ii 1917, p. 687) is of interest:

> It is a drawback to the experimental method, as practised on lines of Baconian induction, that anyone may make a few random experiments and with the results lay some sort of claim to general attention. Hence we should preserve a carefully critical attitude towards claims to medical discovery until some circumstance evinces the likelihood of some truth in them. Lately (Zeitschrift für Sexualwissenschaft, Aug., 1917) the physiological work of Steinach, Foges, and Lode has come in for repeated discussion. Steinach described having changed the sexual disposition of small mammals by implanting, as the case might be, an ovary into a young male or testicular substance into a young female. When the necessary operations were successful the treated animals in their behavior showed reversal of the natural conditions, males attempting to mate with males and females with females. But (a very important point) such was the case only if the young animal so treated had been first deprived of its own primary reproductive gland—i.e., if it had first been castrated or spayed—otherwise the implantation had no feminizing or masculinizing effect. It was, in short, as though a clear field was necessary for the exogenous influence. Around these findings the theory has been constructed that the products of testicular and ovarian secretion—that is the specific reproductive hormone of the two sexes—are sharply antagonistic the one to the other. Their effect on the brain, from which the sexual impulse proceeds, is described as an 'erotising' one, in the direction of masculinity or femininity respectively. The mode of action is supposed to be bio-chemical. The conclusions want more evidence to back them.[2]

It was during the process of some studies on sexual modification in rats and guinea-pigs that Prof. F. R. Lillie suggested the desirability of repeating Steinach's experiments. To Doctor

[2] The article referred to has not yet been obtained by the writer; possibly it has not reached this country.

Lillie I am greatly indebted for many suggestions, abundant material, and a constant interest in the problem. The results agree, in some respects, with those of Steinach, but are not so far reaching as might be expected. This paper contains an account of the observations made on the white rat; a report of similar experiments on guinea-pigs will follow later, as the observations are not yet complete.

MATERIAL EMPLOYED

The white rat (Mus norvegicus) was used for the experiments and the operative procedure described by Steinach has been repeated almost exactly. It appears, however, from differences to be noted later that Steinach must have employed a slightly different strain of rats than those used in these experiments. Animals of the same age and almost invariably of the same litter were selected for the cross transplantations. The method was as follows: A brother and a sister rat were etherized at the same time, and after operative conditions were observed the peritoneal cavity of each was opened.[3] The ovary, after removal and sometimes accompanied by small pieces of oviduct, was cut in half with scissors to aid in the establishment of a vascular connection. A piece was placed on either side of the midventral line of the male between fascia and the abdominal musculature or imbedded more deeply in the substance of the muscle. The genital cord of the male was severed above the epididymis and both testes removed. Pieces of these were similarily placed in the body of the females and the muscle layer with peritoneum, and the skin were sutured separately. Usually a slight injury of the fascial layer of the external oblique muscle as well as the corium of the skin was made with the point of a knife to aid in the establishment of a vascular connection.

Aside from the homoplastic transplantations with complete removal of the normal gonad, some of the animals were merely castrated and spayed without subsequent transplantation; also,

[3] The abdomen was shaved, treated with Lugol's iodine and alcohol, instruments sterilized in carbolic acid solution, and the table covering, towels, gown, etc., sterilized.

several cases of homoplastic transplantation were made in which the normal gonad remained undisturbed. Parts of fifteen to eighteen litters were used in the experiments, comprising over fifty operated animals. All cases of grafting were not successful, and a few animals died before their mature condition could be studied, but the successful cases were sufficiently distributed to afford a wide range of conditions for study. It has seemed advisable to give in detail the history of one litter and allow this to serve as an example of the general conditions in such experiments. This litter fulfills very adequately the conditions sought from the fact that the transplanted gonad obtained vascular connections and persisted in each operated animal. Furthermore, there is need for constant comparisons between operated animals and the normal ones under similar conditions. The one litter eliminates such ordinary differences as age, number of rats in litter, age of mother, hardiness, etc., and since the conditions are the same separate histories will not have to be repeated.

The litter (no. 6, AIBI) was born September 22, 1917, and consisted of four males and six females. Operations were carried out at the age of 25, 28, and 35 days, and all were cases of homoplastic transplantation with complete removal of the normal gonad, i.e., ovaries were removed and pieces of testis placed subcutaneously and vice versa. Of the ten rats in the litter transplantations were made on three males and four females; unfortunately, however, one female escaped from the cage within three days after operating, which left three castrated males containing transplanted ovaries and three spayed females containing transplanted testis, one normal male and two normal females as controls. On November 12, 1917 (51 days old) these were so marked that each one could be recognized and each was then given a number making possible a complete and separate record of each individual rat during the course of the experiment; the experiment was terminated June 3, 1918, at the age of 254 days. At that time the rats were killed and the grafts preserved for cytological study. Since the different rats will be treated individually, the following numbers will serve to identify them:

Litter 6 AIBI

I. Spayed female with testis graft.
II. Spayed female with testis graft.
III. Normal female (control)
IV. Castrated male with ovary graft.
V. Castrated male with ovary graft.
VI. Spayed female with testis graft.
VII. Normal male (control)
VIII. Castrated male with ovary graft.
IX. Normal female (control).

BODY WEIGHT AND BODY LENGTH

It is unfortunate that the distinguishing somatic characters of the male and the female rat are not more sharply marked than they are. However, the studies of Stotsenburg ('09, '12, '13), King, and others[4] show that within certain limits the growth curves (body weight) and the body lengths normally afford criteria for a distinction between the two sexes. Steinach hsa placed considerable emphasis upon these weight and body-length relations of his feminized males and masculinized females as being indicative of maleness and femaleness. It is the opinion of the writer, however, that such slight differences in weight are but poor criteria of maleness or femaleness under abnormal conditions. It is true that the normal curve of growth for male rats is considerably above that for females, but it is decidedly unreliable to choose two or three rats at random and classify them sexually on the sole basis of weight; there is too much tendency for variation even among rats of the same litter for it to be reliable. Also a slight pathological difference may produce a relatively great change in weight even though the rat is in apparently good health. Too many operated animals are required to make the factor a convincing one. But of more importance than this, perhaps, is the fact made known by Stotsenburg that early castration of male rats does not influence the subsequent growth curve, while early spaying of female rats resulted in an increased growth curve over that of the normal female

[4] Complete references of work done on rats have been compiled by Donaldson in a book 'The Rat' (Memoirs of The Wistar Institute of Anat. and Biol., no. 6, 1915).

of 17 per cent, 24 per cent, and 30 per cent in three lots observed. The tendency of growth in the total absence of gonads would then be to equalize the weight of the two sexes, but whether this condition would actually be realized is uncertain. At any rate a spayed female with grafted testis would increase in weight above the normal for females not because of the testis, but because of the absence of the ovary. These factors alone would tend to make the weight of an animal a very unsatisfactory test of maleness or femaleness.

TABLE 1

ANIMAL		66 DAYS	80 DAYS	108 DAYS	138 DAYS	165 DAYS	192 DAYS	261 DAYS	BODY LENGTH, AGE 261 DAYS
		grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*cm.*
I	Female with testis.....	75	92	119	137	149	143	165	18.0
II	Female with testis.....	95	93	128	158	179	177	157	17.5
III	Female (normal).....	95	99	127	147	125	132	145	18.0
IV	Male with ovary.....	80	99	137	158	173	164	[1]	[1]
V	Male with ovary.....	97	106	133	150	159	160	180	18.5
VI	Female with testis.....	88	92	124	159	177	172	185	18.5
VII	Male (normal).....	102	109	139	166	180	190	235	20.5
VIII	Male with ovary.....	89	104	138	160	168	166	193	19.25
IX	Female (normal).....	100	99	125	195	132	160	175	17.5

[1] No. IV, killed at age of 238 days.

Many factors also enter in that tend to discount the apparent specificity of length as a determinant of sex. Stotsenburg's early spayed females were found to increase in body length over that of the normal females. Here again, if these animals had possessed transplanted testis their increase in length could not be considered as a result of the secretion of the testis, but rather of absence of the secretion of the ovary. Nevertheless, to obtain whatever evidence possible a careful series of weights has been kept on the litter in question and is given in table 1. At the termination of the experiment the total body length of each was recorded.[5]

[5] The length of the body and tail, often employed, could not be accurately measured, as a small piece of the tail had been removed from some of them as a distinguishing mark for those individuals.

Thus it will be realized that these physical factors (weight and length) are very poor barometers of the conditions here represented, that of an intersex condition. If, for instance, rats V and VIII (males containing growing ovaries) be compared with VII (a normal male), those containing the ovaries are both lighter in weight and shorter in body length than the normal male, but do not fall to the level even of the heaviest female (IX). We may perhaps, with all justice, refer this decrease to the presence of the ovary. Also if the females containing testis tissue (I, II, and VI) be compared to the mean weight of the two normal females (III and IX) there appears, only in case of rat VI, an increase in weight which by no means approaches the weight of the normal male; and had only female IX been used as control there would be an actual decrease in comparison of I and IX. The former (I) having had the ovary removed should have been heavier than the latter (IX) which had ovaries present.[6] The question also arises whether we should refer these weight modifications to a variation in the intensity of the sexual condition, or whether they may not be merely the result of disturbances in the regulators of metabolism which we know may produce variations. It may be possible that the elimination of some secretion of other glands may affect the final result as well. If this were true, surely we could not consider this secretion as a factor in determining the sexual condition of the animal.

Weight and length are then very unsatisfactory criteria for determining the changes associated with cross transplantation of gonads.

HAIR, MAMMARY GLANDS, SKELETAL CHANGES, FAT DEPOSIT

Steinach has used a few other criteria as tests for the result of the sex hormone (Pubertätsdrüse) in its powers of modification, but the writer also finds it impossible to consider these as valid support for the hypothesis.

[6] Some experiments under way at the present time indicate a potential weight difference in the two sexes that appears to be independent of the gonad. Even though these experiments are not yet complete, the indication is that early spayed female rats, though they increase in weight over that of the normal females, do not reach to the height of the growth curve of the castrated males.

It is possible that the differences of the male and female hair coats of Steinach's rats were more pronounced than in the strain used in these experiments.[7] It is true that a slight difference can be noted in normal healthy white rats of the same age. The male hair coat appears slightly rougher, the hair being a little more coarse than that of the female; this in a general way gives a softer, smoother appearance to the female than to the male. But this also is subject to so many variations that it is decidely unsafe to use it as an indicator. The variations at different ages are considerable, and a slight metabolic disturbance also gives entirely different appearances to the hair. Numerous instances have been noted in which the female coat was rougher in appearance than that of the male. Indeed, the writer has often found it entirely impossible to choose the males and females from a cage of normal and apparently healthy mixed rats by this means alone. This being true, it would be entirely impossible to note the changes in an intersex condition and to place properly these changes as quantitative determiners of a modified sex condition. If one were a decided advocate of the idea, it would be a simple matter to record differences that would support the hypothesis. It is possible, however, that Steinach's material showed greater differences than the rats used for these experiments.

In relation to mammary glands Steinach has already pointed out the fact that rats offer very poor material for study of their changes. The primordial teat is not produced in the male so that little influence from the implanted gonad upon the primordial mammary gland can be seen.[8]

Steinach ('12) has reproduced radiograms of feminized male rats to show the difference in size of the pelvis between these and normal or merely castrated male rats. These radiograms show very clearly the comparatively small size of the pelvis in feminized rats, but they also show, to a like degree, the reduction of

[7] Steinach seems to have used partly wild rats, partly tame (white) ones, and crosses between these.

[8] Guinea-pigs afford much better material for study of possible changes in the mammary gland due to internal secretion of gonads; experiments are now under way on these animals and will be reported at a later date.

all the other bones in the body. No x-ray examinations of the pelvis of the modified rats have been made by the writer, but it appears that probably these characteristics, also, are not specific nor distinctive. In a rat of smaller size one would naturally suppose that the pelvis would be smaller as well as all other bones of the body. And it would seem probable that the condition of intersex, as one encounters it in these cases, would present the same difficulties for discrimination as would weight, length, hair coat, etc.

The fat deposit featured by Steinach is a poor indicator of sexual conditions. It is generally true that the tendency for fat accumulation in the normal female rat is more pronounced than in the male. For this to be constant even in the normal condition presupposes a continued, uniform metabolic condition. The question of intergradations in sex again arises as well as the difficulty of recognizing the quantitative amounts of the fat deposit. To illustrate from this litter: Rats I and VI showed a greater amount of fat deposit than did rat no. V[9] but the two former rats possessed implanted testis,[10] while the latter possessed the implanted ovary, and the fat deposition should have been reversed. Rat VIII, on the other hand, possessed more fat deposit than either I or VI, which should be the case if only the implanted glands were to be considered. This affords us little evidence for or against the assumption of a modification following implantation of the corresponding sex gland.

OBSERVATION ON BEHAVIOR

The behavior of these rats has given more evidence to support the idea that the sex gland regulates the characteristics of the animal than any other set of characters which has been observed.

A. Feminized males

These behavior observations were carried out both while the animals observed were in the cage with other members of this

[9] The amount of fat was not actually determined quantitatively, but merely noted from macroscopic observations.

[10] For conditions of these grafts, see section on microscopic observations.

litter and while separated for observation. It is beyond question that the early castrated male rats which have received implanted ovaries display a maternal behavior towards the young. The two normal female controls (III and IX) gave birth to litters of young during the course of the experiment and often the mother with young was allowed to remain in the common cage. It was repeatedly observed that the feminized male rats would enter the nest with the mother or without her, would nestle the young and repeat exactly the behavior of the mother when the young attempt to suckle. If the litter is a large one and the young from seven to ten days old, the mother will assume a peculiar position to enable the young to suckle; the abdomen is arched and both the fore and hind-legs are widely separated as the young wriggle around underneath in search of teats. The reaction is quite characteristic. This reaction was displayed absolutely typically by the feminized males. The normal male rat and the masculinized females are seldom if ever, seen on or near the nest,[11] and apparently they take no interest in the young. The following observations from the note-book will illustrate the phenomenon and its frequency:

April 30, 1918. Normal female gave birth to litter of six young.

May 1. Normal male and masculinized females had been removed from cage leaving mother and three feminized males. Feminized male V showed all apparent reactions of mother—persistently occupied nest of young with mother and apparently young were attempting to suckle, no teats developed, however, and young could not suckle. Fem. male licked young, tucking same under him, when attempt to remove him from young would attack and bite. Evident mother instincts, would arch abdomen for young to attempt to suckle. Prof. Lillie sees behavior.

May 2. Observed several times during day (normal male now in cage), feminized male IV several times on nest with mother—it lies down with young allowing them to search for teats, arching abdomen as they work around in search of teats. Normal female (mother) had to lie across body of fem. male to get to young, three suckling mother,

[11] The cage in which the litter was confined was 26 x 18 x 12 inches, made of galvanized wire, sides and top, and a movable bottom. The nest was made from paper torn up by the mother and placed in one corner of the cage. When the mother leaves the nest, especially during the early life of the young, she almost invariably covers it with small loose pieces of paper from the edge.

three attempting to suckle fem. male, remained so for forty-five minutes changing position slightly if disturbed but immediately lying down again with young searching around underneath. If young are displaced from nest—mother immediately picks them up in mouth and returns them to nest—fem. male not observed to return them to nest but allows them to remain at the edge where placed. Normal male never seen in nest with mother when latter is suckling.

May 6, 2 p.m. Fem. male IV found on nest with young, on all fours with abdomen arched and young attempting to suckle. Thought at first was mother, reaction so characteristic, but examination showed mother away from nest. Reaction could not have been different in mother, so characteristic, no question whatever as to appearance of same. Young of course could find no teats but were trying very hard. Best and most conclusive reaction yet observed.

3.30 p.m. Feminized males V and VIII removed from cage leaving fem. male IV, normal male and mother. Mother on nest: IV and n/male in corner of cage opposite nest, n/male almost invariably occupies this place and has never been seen to show any interest in young.

4.30 p.m. All three old rats in end opposite nest, young covered.

5.30. All three old rats in end opposite nest, young covered.

6.00. Same.

8.00. Mother and IV on nest, young attempting to suckle both, both removed from nest—n/male not near nest.

8.30 p.m. Mother had covered young, all three old rats away from nest.

9.30 p.m. Fem. male (IV) on nest with all six young underneath abdomen attempting to suckle, abdomen arched, legs spread, all young searching for teats; absolutely normal female reaction. Mother and n/male away from nest, IV removed from nest.

May 7, 8 a.m. Mother in nest with three young suckling, IV in opposite corner of cage attempting to suckle two young—nest had been torn up during night and one young one nestling under n/male but he showed no reaction to it and was only asleep. Placed all young in nest with mother.

11.00 a.m. IV and mother both on nest, mother suckling four young, IV covering two. Norm. male in end opposite nest. IV taken out of nest—five minutes later IV had returned to nest with mother and young, n/male in opposite end of cage. IV again removed from nest, and mother occupied it.

11.45 a.m. Mother and IV on nest with young, one young under IV, n/male in end opposite IV removed from nest.

2.00 p.m. Mother suckling young, IV and n/male in opposite end of cage. Mother removed from nest.

2.40 p.m. IV on nest with young attempting to suckle, removed from nest. n/male in end opposite.

3.15 p.m. IV on nest with young, n/male in opposite corner of cage, IV removed from nest.

3.30 p.m. Mother on nest, IV and n/male in opposite corner.
4.00 p.m. Feeding.
5.30 p.m. Young covered up in nest—all three old ones away from nest.
8.45 p.m. Same.
May 8 a.m.
8.00 a.m. Mother and IV both on nest, IV removed, n/male in opposite corner.
9.30 a.m. Mother and IV on nest with young, n/male in opposite corner. Mother removed from cage, leaving in cage only fem. male (IV) and n/male. IV removed from nest.
10.00 a.m. IV on nest with young, watched reactions for fifteen minutes with Prof. M. M. Wells. IV trying to suckle young—abdomen greatly arched for young to get under, hind legs spread apart when little ones approach that region of abdomen from beneath, changes position slightly as young search from place to place for teats. Proclaimed by Prof. Wells as non-questionable maternal reaction. Young displaced from nest, IV replaces them—picks up young in mouth—suddenly picking up one at a time carries four to opposite end of cage placing them at side of sleeping n/male whose reactions are wholly passive—young begin to crawl under him but he continues to sleep. IV covers remaining two in nest, remain so for five minutes. Young taken from under n/male and placed in center of cage, IV comes off nest, picks up all and returns them to nest, IV disturbs n/male apparently in search of young intrusted to his care, pushes him out of corner. IV moving about cage—mother returned to cage.
May 22. Litter in cage 37—cage contained normal male, normal female, two castrated males, two spayed females.
Observed many times daily for twelve days (until young would leave nest) never was normal male, castrated male, or spayed female seen near young which were in end of cage in nest made by mother. Were never seen to give any attention to young in period of twelve days observed.

Two points in particular are established by consideration of such a set of observations, especially those made at intervals over a period of two consecutive days: these are, 1) that the feminized male (in this case rat no IV) does not merely display a sporadic interest in the young rats, but that it is a continued interest and apparently as characteristic as that of the mother, and, 2) it gives not only a comparison of the feminized male behavior and that of the mother, but it also brings to attention very forcibly the absolute passive reaction of the normal male. Also the series of observations continuing over twelve days fails

to reveal the slightest interest displayed by either spayed females or castrated males.[12]

B. Masculinized females

In order to observe better the reactions of the masculinized females, these were placed alone in cages for a day or so before subjecting them to the experiment. The various rats were then put into the cage and the reactions of the masculinized female noted.

Practically any two strange white rats placed together in the same cage immediately show interest in each other, and whether they are two normal males, two females, or male and female they almost at once begin to nose around the external genitals of each other, and many times if they are both males a fight begins. These reactions are general for practically all rats when placed together. But in case the two are a normal male and a female in heat, the act of copulation begins immediately and, though of very short duration, may be repeated a great number of times.[13] But in all the writer's experience among the rats of the colony used (amounting to several hundred) he has never observed a normal female attempt to imitate the male in the act of copulation. One of the most, perhaps the most characteristic feature of the whole process of copulation in regard to the male, is that after each attempt almost invariably the male assumes immediately a position that allows him to lick the copulatory organ before the next repetition.

It is a very interesting fact that the masculinized female would attempt to imitate the male in the act of copulation in an absolutely typical set of reactions. Of course, no male copulatory

[12] The spayed and castrated rats of cage 37 were at this time over six-months old and had been operated on at the age of about thirty days. These rats normally would have been sexually mature.

[13] The males are not especially keen discriminators, for if a female in heat is placed in the cage with several males the excitement is very great and repeatedly results in one male attempting to copulate with another. This attempt is also often made if a male is placed in a cage with a single male, especially so if a female in heat has just been removed from the cage of the male.

organ had developed, but despite this fact, each time the attempt was made the masculinized female repeated exactly the male behavior by licking the normal position of the male organ. The following extract from my notes represents the type of behavior:

May 29. Normal female in heat, placed in cage with masculinized female (rat I). Mr. F. L. Dunn and the writer watched four unquestionable attempts at copulation, each time masculinized female licks region of penis of male, same as normal male reaction, though no penis is present. Not so enthusiastic as normal male, repetition not so frequent. Same normal female in heat placed in cage with an early spayed female on which no transplantation of testis had been made—no attempt at copulation.

June 3. Normal female in heat, placed in cage with masculinized female (rat I)—reactions perfect and characteristic of norm. male. Copulation attempted eight or ten consecutive times with only short intervals between attempts, masc. female entirely as enthusiastic as norm. male—each time licks external genitals as does norm. male, before next attempt. Normal female taken from cage for fifteen minutes, returned to cage again. Copulation attempted instantly, just as normal male would have acted. Copulation attempted time and time again, absolutely normal reaction.

Spayed female without testis showed none other than passing interest toward female in heat, no attempt whatever to imitate act of copulation. Same true of old female, did not attempt to copulate.

There is absolutely no question as to the occurrence of these reactions, for they could not be more characteristic had the female in heat been placed in a cage with a normal male. The normal female reacts also in an absolutely typical manner towards the masculinized female, just as she would have acted had the masculinized female been a normal male and capable of fulfilling the act of copulation. And it is significant that, so far as the writer has observed, neither a normal female in the height of vigor, an old female, nor yet an early spayed female that had not received the testicular transplantation has ever been seen to attempt the act of copulation by assuming the rôle of the normal male rat.

It may be remarked that the only psychical reactions that appear to be of value to the writer are those illustrated above, those of the sexual reactions of one rat to another and the ma-

ternal behavior. Steinach has described at great length the docility of the normal female rat (does not fight, is easily handled, not so apt to bite or to resist handling, etc.), but here again the variations are too great to be of any practical value. Many females of this colony used are decidely more pugnacious than males. In several cases, these, after repeated handling would bite, scratch, and resemble any other than a meek and mild-tempered female, and at the same time the males show entirely as mild and even-tempered disposition as any female of the colony.

As for the sex reactions, it is true that, in cattle especially and in some other animals, the females often attempt to imitate the rôle of a male; but among rats this tendency has, so far as known, never been observed. As noted above, female rats have been placed in cages with normal females, early spayed females, and old females (each of these having been in isolated cages for some days), but in no case have they attempted to imitate the male. Aside from the general reactions exhibited by any two strange rats when placed together, the reactions except in cases of transplanted testis, have been negative.

MICROSCOPICAL OBSERVATIONS

The litter from which most of the data for this paper has been taken afforded excellent material for these considerations on account of the fact, mentioned previously, that one or more of the transplanted gonads were retained for the 225 days between the time of operation and the termination of the experiment.[14] The animals were killed with ether and the transplanted glands removed from the place of growth and development; the tissue was killed in Bouin's fluid, sectioned, and stained with haematoxylin and eosin.

The young ovary successfully transplanted into a young male animal persists and undergoes its differentiation in quite a nor-

[14] The blood circulation in these transplanted pieces of tissue was established in different cases, either principally through blood connection with vessels of the muscles or from cutaneous vessels in the superficial fascia.

mal manner.[15] Inasmuch as the ovary at the time of transplantation was cut into two pieces, and these probably never of the same size, it was impossible to ascertain the amount of growth in any specific piece. The stroma tissue is quite characteristic of the normal ovary. Both mature and immature Graafian follicles are abundant and some contain ova apparently ready for ovulation. That ovulation really does occur is shown by the presence of a few corpora lutea, some of which still contain a blood clot in the center. Many Graafian follicles indicate an evident tendency for degeneration, and atretic follicles are often found in the preparations. Reference to figure 1 will convince one that the ovarian cortex, stroma, and contained follicles as well as the medulla of the ovary are retained in apparently a normal condition. This section is a piece of the original young ovary placed subcutaneously in the male at the age of thirty days and allowed to grow until the animal was killed 230 days later. Vascular connections may be established either from cutaneous blood-vessels or in case of a deeper transplantation by vessels supplying the abdominal muscles. This graft was imbedded in the superficial fascia. The section passes through the ovum of two mature follicles and each contains a very distinct nucleus. Figure 2 (from same graft as fig. 1) shows not only the more mature follicles, but a young follicle as well as a corpus luteum. The graft from which these two sections were prepared had persisted throughout the entire period of the experiment in almost a normal condition. It was physiologically active, since the germinal epithelium shows an apparently normal condition, young follicles are present also mature follicles evidently almost ready for ovulation, and corpora lutea are present, showing that a previous ovulation has taken place. The condition of this graft can represent the general condition of the persisting grafts, since no other features worthy of mention have been noted in other grafts.

[15] In several cases of transplantation of both ovaries and testis, more especially testis, the glands failed to persist and underwent resorption, leaving little if any traces of the original implantation.

The implanted testicular tissue does not persist in the same degree of normality as does the ovary, and in some cases it has

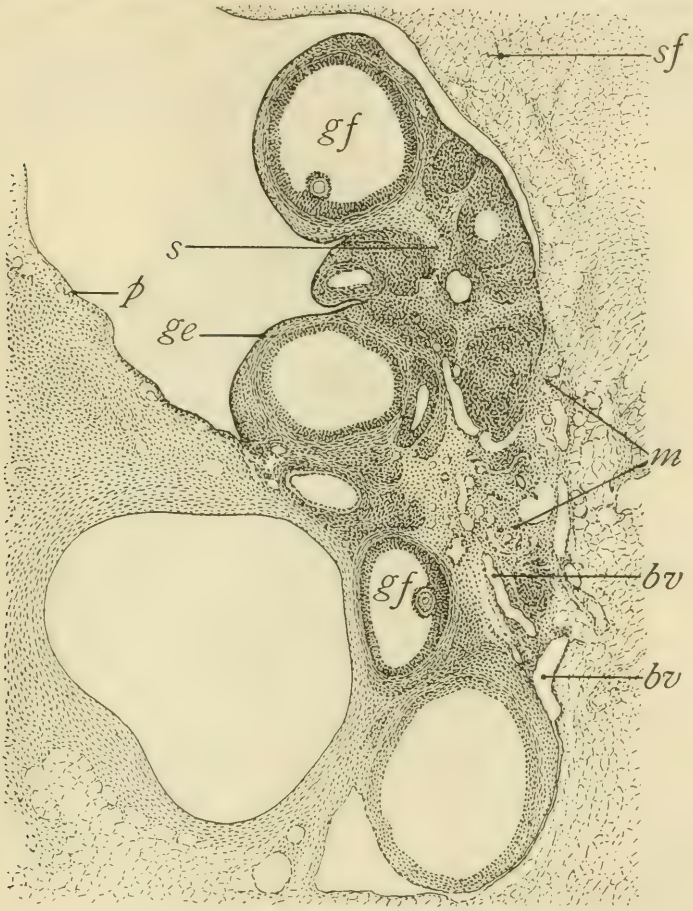

Fig. 1 [16] Section of an ovarian graft in superficial fascia of male rat (6, AIBI-V). Rat 30 days old when graft was made; growth removed after persisting for 225 days. *bv.*, blood-vessels; *gf.*, Graafian follicle; *ge.*, germinal epithelium; *m.*, medullary region of ovary; *p.*, peritoneum of female transplanted with ovary; *s.*, stroma; *sf.*, superficial fascia.

failed to persist at all. In the majority of cases the tissue was placed subcutaneously in the female near the midabdominal

[16] Drawings made by Kenji Toda.

line. Usually the seminiferous tubules are found scattered about either in the muscle tissue or in the fascia above and are not bound up into a compact mass; figure 3 (a section of a 225-day testicular graft in female 6, AIBI-I) shows the dispersed condition of the tubules imbedded in muscle. In figure 4 (same age graft as in figure 3, but taken from female 6, AIBI-II) part

Fig. 2 Section from same ovarian graft as figure 1. *bv.*, blood-vessels, *cl.*, corpus luteum, *ge.*, germinal epithelium, *gf.*, Graafian follicle, *s.*, stroma.

of the epididymis as well as the degenerated seminiferous tubules is present. These tubules, as Steinach has described, are decidedly different from those found in a normal testis; they contain only an irregular lining of cells of large size (interpreted as Sertoli cells). Spermatozoa and spermatocytes are entirely absent. The lumen of most of the tubules is filled with a reticular substance possibly representing products of degenerated

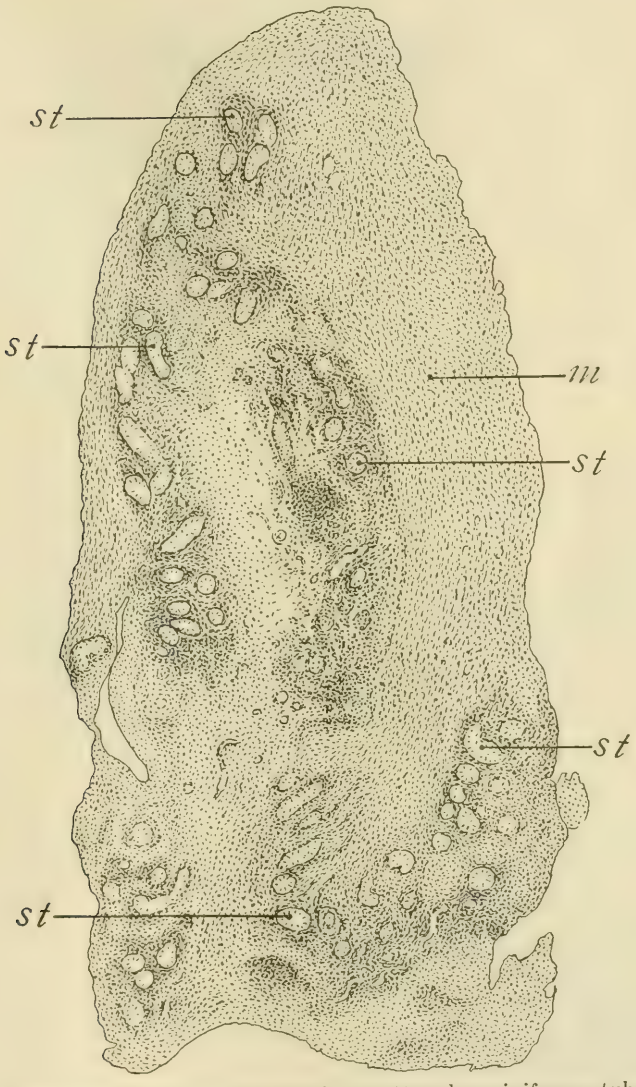

Fig. 3 Section of testicle graft showing scattered seminiferous tubules in the abdominal muscles of female rat. Graft had persisted for 225 days before its removal from the female (6. AIBI-I). Tissue transplanted from male to female at age of thirty days. *m.*, muscle; *st.*, seminiferous tubules.

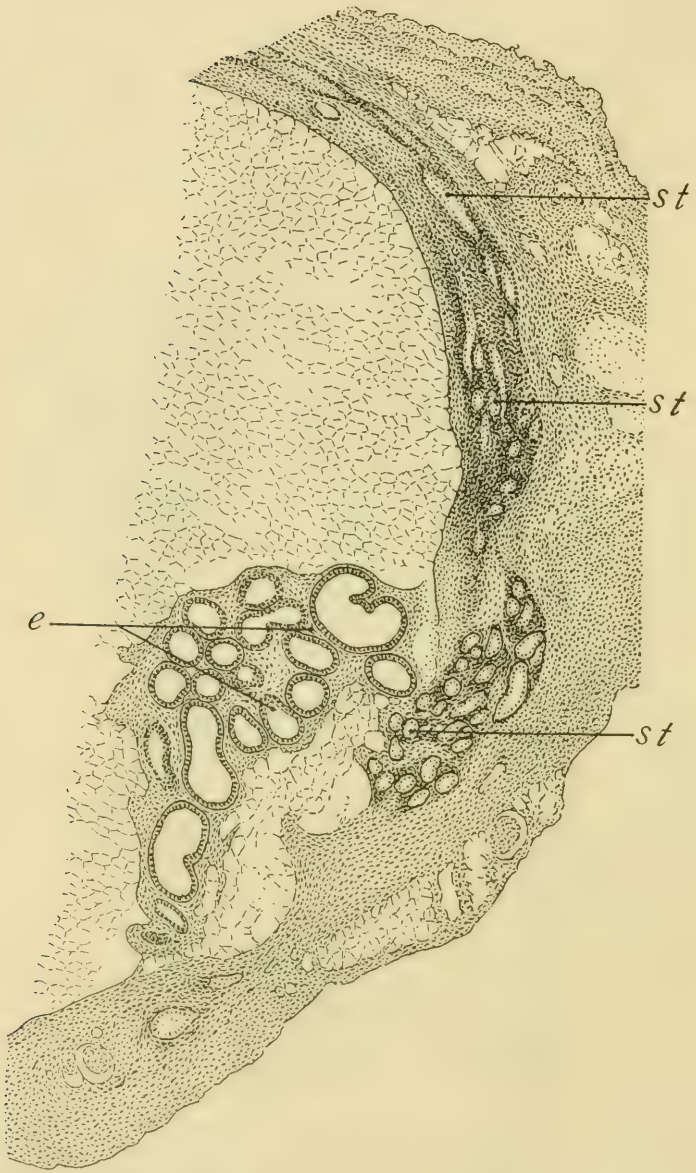

Fig. 4 Section of testicle graft showing seminiferous tubules and part of epididymis, from muscle and fascia of female rat (6, AIBI - II). Graft same age as figure 3. *st.*, seminiferous tubules; *e.*, epididymis.

cells. Figure 5, a more highly magnified part of the graft from female 6, AIBI-II, shows the condition of the cells that remain within the tubules as well as the large number of cells between the tubules. The latter evidently represent the interstitial gland described by Steinach and supposed by him to be the seat of the production of the hormone of the testis. Between the tubules are usually to be found a great number of leucocytes scattered indefinitely throughout the tissue, but the significance of these is not entirely clear.

The writer purposely refrains from an elaborate description of the cytological findings, as Steinach has discussed them adequately. The figures included will enable one to realize the condition of these pieces of transplanted gonads.

DISCUSSION

These observations corroborate parts of Steinach's experiments and tend to support very strongly his ideas of the transforming power of the gonad of one sex over, at least, the psychic nature of the opposite sex. While the writer is entirely unable to interpret the observations of the possible somatic differences of these evidently modified rats as a distinction of maleness and femaleness, nevertheless the psychic behavior of the animals, absolutely distinct in itself, lends great weight to the idea of a transformed sexual nature.

On the somatic side the early spayed female rat with implanted testis increases in weight, but it has been shown that the testis has no influence upon growth, but that elimination of the ovary does allow a relative increase in weight; the same is also true in regard to body length. The presence of the ovary tends to retard the growth of the animals, either male or female, but it is very difficult if not impossible to interpret these changes intelligently in relation to a modified sexual condition. The testis is entirely without effect in this regard.

Neither the hair coats, fat deposition, nor temperamental behavior outside of sexual reactions and maternal instincts appears to the writer to be of any deciding significance, and the size of the pelvis and other bones, for reasons already given, appear at

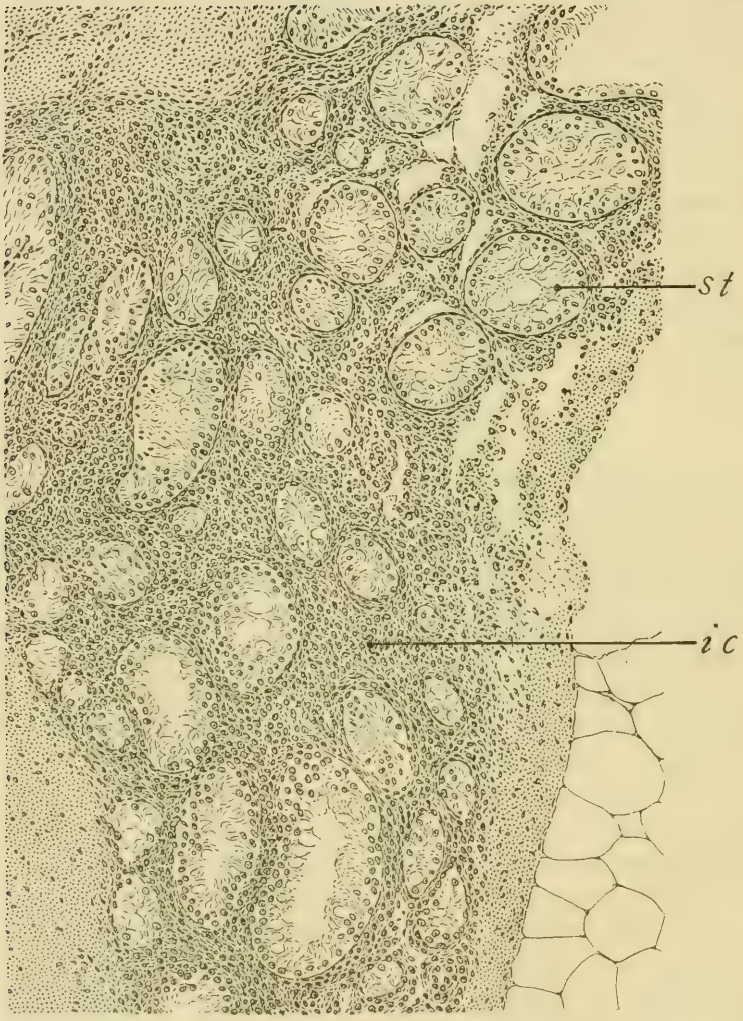

Fig. 5 Section of testicle graft (same as fig. 4) more highly magnified to show interstitial cells, and degenerated condition of tubules. *st.*, seminiferous tubules; *Ic.*, interstitial cells.

best to be very poor criteria of maleness and femaleness. The negative influences, such as failure of the penis and seminal vesicles of the male rat to grow, are nothing more than we could expect in any castrated form. It has been shown repeatedly that many structures of this kind depend upon the presence of the testis for their growth and development. And since the primordium of these are absent in the female, we could not expect their development.

Guinea-pigs as well as rats afford good material for these considerations only from the ease with which they are handled and with which they withstand operation, but they afford very poor material from which to draw demonstrable conditions and definite conclusions in regard to sex modification of this kind. They possess no distinct sexual differences, aside from the internal and external sexual organs, that will specifically classify them as a male or female, and hence are decidedly inadequate for experimental purposes to decide the question at hand.

The writer purposely postpones further discussion of the bearing of these results until the observations on guinea-pigs are complete.

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Resumido por Donald Walton Davis, por el autor, Herbert W. Rand.

Multiplicación asexual y regeneración en Sagartia luciae Verrill.

Sagartia luciae se reproduce asexualmente por medio de una bipartición en sentido aboral-oral, seguida de regeneración. El plano de esta bipartición es vertical y tiende a ser perpendicular al eje mayor de la boca, tendiendo a cortar los endoceles a preferencia de los exoceles y en este caso suele cortar los endoceles completos a preferencia de los incompletos, seleccionando los no directivos más bien que los directivos. Los productos de esta bipartición son generalmente desiguales. Después de la bipartición los bordes de la pared del cuerpo se encuentran y fusionan. Los del esófago se reunen también y en el centro de la región fusionada se desarrolla invariablemente un sifonoglifo. A cada lado de este plano directivo nuevamente establecido se regeneran dos pares de mesenterios completos no directivos, siempre que el mesenterio originario más próximo sea incompleto; pero si es completo y no directivo se regeneran un par de mesenterios completos no directivos más un mesenterio completo único, el cual se dispone en pareja con el mesenterio impar originario para formar un par no directivo. Los mesenterios incompletos se regeneran conservando las posiciones caracteristicas. El autor describe el orden de desarrollo de los nuevos mesenterios; en relación con ellos se desarrollan nuevas bandas anaranjadas conservando su posición característica. La regeneración añade una serie de estructuras bastante definida y fija que no guarda relación alguna con el tamaño inicial y la forma de los productos de la bipartición. Antes que la regeneración se haya completado pueden llevarse a cabo otras biparticiones. Los individuos resultantes son, por consiguiente, diferentes en extremo con relación al número de sifonoglifos, mesenterios y bandas de color anaranjado. Sagartia luciae es, probablemente, fundamentalmente hexamérica. La escasez de individuos regulares hexaméricos depende probablemente de este proceso de bipartición y regeneración que probablemente es el medio más efectivo para mantener o aumentar la población de una región determinada.

Translation by José F. Nonidez
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ASEXUAL MULTIPLICATION AND REGENERATION IN SAGARTIA LUCIAE VERRILL[1]

DONALD WALTON DAVIS

FORTY-TWO FIGURES

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[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, No. 318.

INTRODUCTION

This paper deals with the processes of fission and regeneration and their variations in the sea-anemone Sagartia luciae Verrill, together with the relations of these processes to the distinguishable morphological types and to the life history of the species.

Many descriptions of isolated instances of multiplication by asexual methods in Actinians have been published. Among these are somewhat detailed accounts of the external aspects of the process of fission. The present paper stresses the internal phases of this process, to which little attention has heretofore been paid. A few studies have been made of the regenerative processes following the separation, but no connected account of the external and internal features of division and regeneration in a single species has hitherto appeared. Moreover, in species of Aiptasia, where our information is most nearly complete, the processes are markedly different from those in S. luciae.

Variations in the details of form of Actinians have long been the subject of passing comment or of more or less serious study. That these variations, more specifically variations in the number of siphonoglyphs, are related to processes of reproduction was first suggested for Metridium dianthus by Parker ('97) and has been demonstrated in that species by Hahn ('05). A similar suggestion as to S. luciae was made by Davenport ('03). This suggestion has been confirmed in the observations here reported. Owing to the presence in S. luciae of external markings which are not found in other anemones and which give evidence of fundamental internal structures, it has been possible to determine the relation of the processes of asexual reproduction to these internal structures. The significance of studies of the asexual processes of S. luciae is increased by failure to obtain thus far any stage in its ontogenetic development.

For various reasons, more complete knowledge of the life history of this species is desirable. Its appearance at New Haven about 1892 has been reported by Verrill ('98); its rapid increase and gradual dispersal have been described by Verrill and by Parker ('02). It is now the most abundant sea-anemone

found between tidemarks on the coast of southern New England. Prof. H. B. Torrey called my attention to its occurrence in San Francisco Bay as early as 1906. Hargitt ('14) refers to a report of its presence at Plymouth, England, in 1908, and records its identification at Naples in 1911. It may be collected at any season and lives well in the laboratory for long periods. A few liters of sea-water is all that is necessary to maintain many specimens indefinitely; a finger-bowl with a liter of water for changes serves well for a few. It may be shipped in moist seaweed without injury. It is therefore available not only at the seaside, but wherever desired.

I wish to express my hearty thanks to Prof. W. E. Castle for his kindness in directing the work in its earlier stages, and to Prof. H. W. Rand, whose encouragement and suggestive criticisms have been most helpful in the later stages. The work has been done chiefly in the Zoölogical Laboratory of Harvard University and in the Woods Hole Laboratory of the United States Bureau of Fisheries. To the authorities of these institutions I am indebted for the facilities generously afforded. To these and to others who have in various ways lent aid, I here make grateful acknowledgment.

DESCRIPTION OF MATERIAL

Sagartia luciae Verrill is one of the smaller sea-anemones, but highly variable both in size and in relative dimensions. While each individual has its particular tendency toward a more elongated or a broader condition, the same individual under different circumstances may be elongated and narrow or short and thick, and owing to the varying amount of fluid contained in its cavities its total bulk changes greatly from time to time. In still water the animal tends to elongate and, in a state of normal expansion, shows a length of column one and one-half to three times its diameter. In strong currents the tendency is toward a shorter and thicker body, the length of the body being even less than the diameter. Specimens attain rarely a length of 20 mm. or a diameter of 15 mm., but in no case are

these extreme dimensions approached simultaneously in the same individual. In the majority of specimens the more extreme measurement does not exceed half the stated maximum.

The ground color of the column is olive-green. A variable number of orange or yellow stripes extend along the whole length of the column from the oral disc to the base. Less conspicuous vertical lines along the column, darker than the ground color as seen by transmitted light, mark the attachment of the mesenteries.

In oral view the siphonoglyphs, commonly two, may be distinguished with the aid of a hand lens. Except in very small specimens or in early stages of regeneration, each siphonoglyph is clearly marked by a chalk-white bar which extends from the siphonoglyph radially across the disc. The white bars appear with approximately normal prominence in the photographs shown as figures 1 and 2.

In its internal arrangements this anemone follows the general Hexactinian plan, yet with great variation in detail. The number of siphonoglyphs and associated pairs of directive mesenteries is commonly two, but may be one, three, or rarely four (tables 11 and 13). In one specimen five pairs of directive mesenteries were noted. The number of pairs of complete non-directive mesenteries varies approximately from four to eleven; although specimens may frequently be found, at an early stage of regeneration when the new mesenteries have not become established, with less than four pairs of non-directives in evidence.

The form resulting from ontogenetic development has not been determined with certainty, but is probably (p. 218 ff.) a regular hexameric one with two siphonoglyphs, two pairs of directive mesenteries and four pairs of non-directives (fig. 26). In the animals studies, one, two, three, or even four (fig. 25) cycles of incomplete mesenteries are present. These are quite regular in number and arrangement in an area formed at one time, but vary greatly between newer and older regions of a regenerated animal.

I shall use the terms 'endocoel' and 'exocoel' in their accepted significance, the former indicating a space between the members

of a pair of mesenteries, the latter a space lying between two adjacent mesenteries not members of the same pair. It will be convenient to distinguish between the endocoels partially enclosed by complete mesenteries and those bounded by incomplete mesenteries. The former I shall call 'complete endocoels,' the latter, 'incomplete endocoels.' Obviously, the spaces between members of a pair of complete mesenteries may be further distinguished as directive or non-directive endocoels, and incomplete endocoels may be designated according to the cycles of the incomplete mesenteries bounding them.

The white bar on the disc extending radially from a siphonoglyph marks the position of a directive endocoel, with which the cavity of a so-called directive tentacle communicates. Ordinarily the orange stripes seen on the column of the living animal mark all the complete endocoels and the endocoels of the first cycle of incomplete mesenteries (p. 207). In very small specimens these orange stripes may be wanting entirely. In regenerating regions they appear rather late and, so far as has been determined, without regularity (p. 214). In such regions no dependence can be placed upon the number of orange stripes in determining the number of complete mesenteries. By the time the regenerating area, which is at first paler than the older portion, approaches closely to the old region in depth of color, the orange stripes have developed in full number and, almost without exception (p. 213), accurately denote the number of mesenteries of the first two cycles.

The specimens used were collected at various places and at all seasons. They were kept in the laboratory, for as long a time as desired, in small dishes of sea-water. When first taken to the laboratory the water on them was changed every few days. After algae became abundant on the walls of the dishes, changes were necessary only in order to compensate for loss by evaporation. Three or four times a week the slime which accumulates over the surface of the animals was washed off with a pipette. Feeding was attempted by placing minute shreds of fish or other meat upon the tentacles. This was effective, but very laborious. It was also hazardous, since any particles of food left in the

dishes after feeding or disgorged by the animals tended to foul the water. It was found, furthermore, that the animals thrived quite as well without this attention, doubtless being supplied with sufficient food in the form of minute organisms. Under these conditions specimens decrease in size somewhat at first and after a time become lighter in color. In other respects they appear to remain in perfectly normal condition.

In order to study the internal structures, specimens were fixed and sectioned. The larger mesenteries can be seen in sections cut by hand, but smaller mesenteries are sure to be overlooked if dependence is placed upon that method of examination. My specimens were therefore stained, embedded in paraffin, sectioned, and studied with the compound microscope.

For narcotizing, A. G. Mayer's method of immersion in a 3/8 mol. solution of magnesium chloride has proved entirely satisfactory. After a half-hour in this solution the specimens are thoroughly stupefied and almost invariably well extended. Animals in good active condition tend to expand in this solution, even though they may at first contract from the mechanical stimulation incident to immersion. Unless fine histological fixation is desired, 4 per cent formalin is a convenient and satisfactory killing agent. From this the specimens may be transferred immediately to 70 per cent alcohol. Staining in toto for eighteen to twenty-five hours in Kleinenberg's haematoxylin brings out well the mesogloea, which gives the best evidence of the position of mesenteries and of the longitudinal muscles upon them.

Some of the specimens whose regeneration is recorded divided under observation in the laboratory; others were discovered, either in their natural habitat or in the laboratory, at the close of division or immediately following separation of the parts. Nearly all of the other specimens examined have shown externally some portions of regenerated material.

FISSION AND REGENERATION

The frequent occurrence of fission in this species has been noted by Davenport ('03) and the process has been well described by Hargitt ('14). A very similar process has been described for a closely related form by Torrey and Mery ('04). The process apparently consists simply in the tearing of an individual into two parts by the migration of two portions of the basal disc in opposite directions. The first suggestion of an approaching division is seen in the elongation of the base from its usual nearly circular outline to an oval form. In the course of a few hours or days this elongation becomes more extreme. The whole body becomes flattened closely against the substratum, evidencing a state of extreme tension. Eventually a rent occurs in the base. This rent widens, gradually extends up the column and finally involves the oral disc and the esophagus. Division may result in the production of more than two pieces almost simultaneously; although, as I shall show (p. 173), this occurs really by successive fissions rather than by simultaneous division into three or more parts. I believe that the mouth and esophagus are invariably cut by the plane of fission. There is no suggestion of a division resembling that known as basal fragmentation. Any other method of asexual reproduction than that described above must be very rare in the adult form of this species.

Certain external features of the process of reconstruction following division in S. luciae have been described by Davenport ('03), and in S. davisi by Torrey and Mery ('04).

After fission is accomplished, the two resulting individuals remain in a limp state closely pressed to the substratum, and the torn edges of each slowly roll inward and fuse. Within a day or two the separate pieces, their wounds having closed, acquire the normal upright position and typical cylindrical form, and the tentacles become expanded. For some days after this the region of fusion of the edges shows as a narrow vertical streak of much lighter color than the adjacent old parts of the column. At this stage in less brilliantly colored specimens the new tissue

may easily be mistaken for an orange stripe (cf. fig. 3, 4). In the course of weeks this regenerating part increases both in absolute size and in proportion to the old tissue until finally it forms, in some cases, much the greater part of the polyp. Also the newer region becomes darker until it is no longer distinguishable in color from the older. Meanwhile tentacles and a faint white bar are formed in the new region of the oral disc, and pale orange stripes appear in the new area of the column. A new white bar is at first fainter and narrower than an old one. This is illustrated at *3* in my figure 2, and better by Hargitt's colored figure ('14, fig. 2). The distinction persists for months at least. The new orange stripes, besides being paler, are closer together than the old ones. In most cases these features, especially the closer grouping of the stripes, enable one to distinguish a regenerated region for many months (figs. 36 to 41; also Davenport, '03, figs. 8 to 11, 14), the exceptions being chiefly small and faintly colored individuals or those in which one or more regenerating regions constitute almost the whole of the specimen. Sections show that a new siphonoglyph is associated with the new white bar, and that in time new mesenteries are formed in the sector regenerated in the region of fusion of the torn edges. Even when the external distinctions between old and regenerating tissue are absent, the mesenteries, as seen in stained sections, may give perfectly conclusive evidence that regeneration has occurred and even locate the precise position of the boundary between new and old portions (p. 169 ff).

No obvious change occurs in the older regions in consequence of fission, except in the parts adjacent to the torn edge, which are subject to injury in the course of the division. No old incomplete mesenteries become complete, except perhaps in the rare cases where probably such a mesentery is itself injured and fuses with the esophagus or where the uninjured mesentery unites by chance with a torn edge of the esophagus. Similar unions between other parts sometimes occur, more often in artificially cut specimens than in normally divided ones. Abnormal adhesions of the latter sort commonly fail to persist, whereas the more nearly normal adhesion between a mesentery and the

esophagus apparently remains (p. 171 ff.). Absorption of old mesenteries, so constant a feature of asexual reproduction in some other species, does not occur here (p. 171, 172, 200), except probably in the case of mesenteries torn during division. So far as I can judge, the rate of formation of new cycles of incomplete mesenteries is not altered. No new orange stripes are formed in the old region as a result of division. A general fading of the brighter colors occurs in all specimens kept under the artificial conditions of the laboratory, but this is not noticeably greater in regenerating polyps than in apparently undivided ones. It appears, then, that the form of the older part is not changed in consequence of fission and that the formation of new structures is strictly confined to the region of union of the torn edges, where a broad sector of new material is laid down. The rate at which regeneration goes on is strikingly variable. In the following account the estimated periods must therefore be regarded as rough approximations, from which individual specimens may be expected to diverge widely.

PLANE OF FISSION

In fission of the type described above, there are many planes in which the tear may pass through the column of the anemone. It is my purpose in this section to discover any tendencies of the plane of fission to occupy spaces of one kind rather than another. Part of the material upon which is based my study of such tendencies consists of specimens observed in process of division and subsequently isolated and allowed to regenerate for different periods before being killed. The mesenteric formulas of these animals of known relationships are given in tables 3, 4, and 5.[2] Table 6 presents the formulas of additional specimens available for this study. Before directing attention to the tendencies indicated by these tables it is desirable to make clear the standing of the additional specimens included in table 6. Study of specimens

[2] For interpreting the tables and figures, to which frequent reference is made in the text, an acquaintance with the symbols and general explanation given on pages 232 and 233 is essential.

in all stages of regeneration show that the exact position of the boundary between the old material and that laid down following division can be distinguished, even after a long period of regeneration, by the relative size and development of the mesenteries of corresponding cycles (figs. 11 to 16). In many instances where the precise position of the boundary cannot be determined, the relative condition of the mesenteries, the degree of development of the mesenteric filaments, and the presence of certain types of arrangement of the developing mesenteries peculiar to zones of regeneration indicate with certainty that division has occurred. Thus in figure 15 the mesenteries labeled c^2 and c^3 lag behind the other members of their respective pairs in becoming attached to the esophagus. A similar condition is represented in figure 18 and constitutes the sole evidence that regeneration is in progress. In the latter instance as well as in one half of the specimen illustrated in figures 15 and 16 the exact position of the plane of division cannot be determined. In figures 19 to 24 the condition of the incomplete mesenteries gives evidence that different regions are of different ages without indicating the precise position of the boundary between newer and older parts. It is obvious that only such specimens as reveal the exact boundary between new and old structures should be included in table 6, but not even all of these can fairly be counted. As indicated by the figures just cited, evidence of division commonly persists longest in the incomplete mesenteries. Hence at a late stage of regeneration, following a division which does not involve a pair of incomplete mesenteries, there may be doubt as to whether one or both of the members of a pair of complete mesenteries lying near the boundary belong to the old region. If only one is old, the division was in a complete endocoel; if neither is old, the division must have been in an exocoel. See, for instance, the older regenerating region of the specimen shown in figures 15 and 16, where the mesenteries marked c are probably (but not certainly) old. Such a case cannot be counted, of course. But specimens in a correspondingly late stage of regeneration after a division between incomplete mesenteries, although they do show the precise position of

the plane of division, should also be excluded from a group on which to base a study of the relative frequency of divisions in different spaces. Such a case is illustrated in the newer regenerating region represented in figures 15 and 16. Therefore only such specimens have been included in table 6 as show the characteristic differences between new and old complete mesenteries. This introduces a slight error through the exclusion of such a specimen as that represented in figure 17, which has no old complete mesenteries. Furthermore, since the specimens of table 6 are to be regarded as a random sample, so far as concerns the position of the division plane, there have been excluded from it all animals *selected* for sectioning because of the relation of the new area to the directive plane or to the orange stripes which mark the complete endocoels and certain incomplete endocoels.

It is to be noted that the plane of division is in almost all cases strictly vertical. Occasionally a mesentery is found in an oral or aboral region, but not throughout the length of the column. Such mesenteries, as would be expected, are usually small. That they represent mesenteries torn during fission is probable, in spite of the fact that not a single case has been noted in which both paired individuals show parts of the same original mesentery. Since such partial mesenteries are rare in older parts, it is likely that they either are normally completed by regeneration, or are absorbed. As I have seen nothing whatever to indicate that a torn mesentery ever grows up or down the column, and as there is some evidence (p. 172)that absorption of mesenteries does occur and that regulative processes correct certain abnormal adhesions, it is likely that the partial mesenteries referred to are removed by absorption during regeneration.

Certain other indications of departure from a perfectly simple vertical tear may be mentioned. In pairs 3 and 10 of table 3 there is disagreement between the bounding mesenteries of the related individuals. In no. 3a an incomplete mesentery of the most advanced cycle (I) stands in the position of a mate to a complete mesentery, c, in no. 3b. In no. 10a an incomplete mesentery of doubtful grade, (1) is similarly opposed to a directive, d, in no. 10b. It is entirely improbable that in these cases

the unlike bounding mesenteries were adjacent to each other in the original animal, since pairs of mesenteries of different character are rare. The apparent lack of agreement may be due in some cases to a tearing from the esophagus of mesenteries previously complete or, in a single case (no. 18b, table 5), to the fusion of an incomplete mesentery with the esophagus during closure of the wound following fission. That complete mesenteries should occasionally have their connection with the esophagus broken is not remarkable considering the nature of the process of fission. The possibility of the abnormal union of an incomplete mesentery with the torn edge of the esophagus is supported by the fact that, in early stages of regeneration of cut specimens, atypical adhesions of parts are common. Such adhesions seem much more rare in later stages, indicating that regulation probably occurs. It is conceivable that an attachment of a normally incomplete mesentery with the esophagus might persist even though adhesions of other parts should be eliminated.

There remain four cases (pairs 3, 10, 15, and 22) in which the most probable explanation of the disagreement is the complete elimination of one or more mesenteries. It is possible that this loss of mesenteries is due to the detachment during fission of a minute piece which was overlooked and lost; or it may be that such a piece was partially separated from the larger ones, or otherwise extensively damaged, during division and subsequently absorbed. In support of the latter hypothesis may be mentioned certain mesenteries found in a few specimens not represented in the tables. These are mostly pieces regenerating after being separated by artificial cuts. The abnormal mesenteries are attached to the column wall, but not to the esophagus. They extend through only part of the length of the column. The mesogloea of these mesenteries is thick and stains heavily, but the longitudinal muscles are feebly represented. They are certainly injured old mesenteries and are probably in process of elimination.

Summarizing the occasional irregularities adjacent to the plane of fission, we may say that small mesenteries may be cut in two

by the fission plane and later resorbed; complete mesenteries may be torn from the esophagus and persist as incomplete mesenteries; incomplete mesenteries may adhere to the torn edge of the esophagus and thus appear as complete mesenteries, and one or more mesenteries may be either wholly eliminated by tearing during division or by this process combined with absorption during the early stages of regeneration.

It should be emphasized that the irregularities just referred to are distinctly exceptional. In general the plane of fission is strictly vertical; and, considering the apparently mechanical tearing of the tissues in fission, there is remarkably general agreement between the mesenteries found in the separated parts.

Ordinarily an individual separates into two parts, which regenerate for a considerable period before a second division supervenes. Occasionally one finds instances, such as are represented in table 5, of division resulting in the early formation of three or more regenerating individuals. The case of no. 19 is typical. It was a large diglyphic specimen with twelve orange stripes and giving no external evidence of a previous division. It was found upon Fucus, where the slimy foothold may have operated to prevent division. The presence of well-developed gonads, as shown in the photographs of products of this division (figs. 27 to 32), may also be associated in some way with a delay in fission under natural conditions. Upon being brought into the laboratory, like many other specimens, it promptly migrated on to the glass surface of the container, and in about a week showed an early stage of fission. One week later a division was completed, resulting in one large and two smaller pieces. After three days more the largest part had divided into two. There were then four not very unequal pieces. Thirty-four days thereafter three of the regenerating products of the division were killed, the fourth having been lost. In no. 22 the interval between successive fissions was eighteen days; in no. 17, twelve days; in no. 18, two days. In nos. 20 and 21, as in the first division of no. 19, the observations suggested simultaneous production of three parts. The number of regenerating zones, however, as

indicated in the table, are such as would result from successive simple divisions. The condition of no. 21 is conclusive in this respect, the evidence from nos. 19 and 20 being less positive because of loss of parts. I have found no instance of division of an animal into three parts giving rise to but three regenerating regions, as would be expected in case of a single compound division.

One might expect to find that specimens dividing into more than the usual number of pieces would be shown to have had an unusually high number of old mesenteries. Such, however, is not the case. Only no. 17 possessed, before division, a high number of complete mesenteries. The average for specimens included in table 5, omitting nos. 19 and 20 on account of missing parts, is 13.5 complete mesenteries. The average for specimens dividing into two parts, included in tables 3 and 4, is 16.4. Evidently, then, the close recurrence of fission is not correlated with a high number of complete mesenteries. Indeed, it seems to be related rather to a number lower than the average; and it eventually tends through generations to raise the number of mesenteries higher than does the more common simple division. As will appear from the statements below, multiple fissions are not associated with particular numbers of siphonoglyphs and pairs of directive mesenteries.

Tables 3 to 5 record the divisions of twenty-two polyps. Seventeen of them were diglyphic before division, three were apparently triglyphic, one was monoglyphic and one tetraglyphic. In thirteen cases a diglyphic individual separated into two parts, each receiving a pair of directive mesenteries. One of the diglyphic specimens (no. 17) divided into three parts and another (no. 19) into four parts. In both of these cases one pair of directives passed to each of two of the resulting pieces, and one or two pieces contain no old directives. Another of the diglyphic specimens (no. 16) divided into two parts, one with two pairs of directive mesenteries, the other with none. Still another of the diglyphic individuals (no. 21) divided into three parts, one part with one pair of directives and a single bounding directive, one part with the corresponding bounding directive

mesentery, and one part lacking directives. One of the triglyphic animals (no. 10) divided in a directive endocoel on one side, each of the two resulting individuals receiving a pair of directives and an unpaired directive (in one part the directive on the boundary being absent). A second triglyphic specimen (no. 22) divided into three parts, one receiving a pair of directives, another receiving a single directive mesentery on one boundary, and the third receiving a pair of directives and an unpaired directive. The third triglyphic animal (no. 20) divided into three parts. One part received one pair of directives; a second part possessed no old directives, and the third, which was not successfully preserved, must have received two pairs of directive mesenteries. The monoglyphic animal (no. 18) divided into three parts not through the directive endocoel. Consequently one part possesses a pair of old directives and two parts have none. The tetraglyphic specimen (no. 15) divided into two, giving one part three pairs of directives, the other one pair.

Thus in fifteen out of seventeen cases of the division of diglyphic individuals the plane or planes of division cut the major transverse axis, giving the pair of directives at the extremities of this axis to different pieces. The division of the monoglyphic specimen occurred in a corresponding plane. One of the triglyphic specimens divided along a similar plane as regards two of its pairs of directives, the division passing through the third directive endocoel. In three of the four remaining cases (nos. 16, 21, and 22) some of the directives are regenerated ones whose imperfective development at the time of division may give occasion for the unusual position of the fission plane. The fourth specimen (no. 15) also has some regenerated mesenteries, but the limits of the regenerating zones are obscure, owing, at least in part, to faulty preservation and to sectioning in a somewhat oblique plane. What part the directives play in determining the position of the division plane may only be surmised. This question is discussed below. Here it should be emphasized, first, that the number of directives does not determine the number of parts into which a polyp shall divide; secondly, that in division there may be separated a piece which

before regeneration possessed no directive mesenteries, and, thirdly, that the division plane cuts the directive plane.

We may now consider more specifically how nearly perpendicular to the major axis of the mouth is the plane of division. In other words, is there any tendency toward bilateral symmetry of the structures of the old piece with respect to its directive plane? The simplest cases in which this problem may be studied are those of originally diglyphic specimens with symmetrically placed non-directive complete mesenteries. Eight specimens of this nature are recorded in table 3. Three of them divided in spaces other than complete endocoels. Of two regular hexameric individuals in this class, one (no. 4) divided in two incomplete endocoels forming two pieces, each symmetrical with respect to its directive plane; the other (no. 6) divided in one complete and one incomplete endocoel forming pieces as nearly symmetrical as could result from division in such spaces. A third specimen (no. 1), with eight pairs of complete non-directives, divided likewise through one complete and one incomplete endocoel into two nearly symmetrical parts.

Five originally symmetrical polyps divided in two complete endocoels—assuming that in two cases a bounding mesentery recorded as doubtfully incomplete was really complete at the time of division. Four of these (nos. 7, 11, 13, and 14) were regular hexameric specimens and one (no. 9) was regularly octameric before division. Each of these five divided into two symmetrical pieces. Neglecting the possibility of division in directive endocoels, the chances are even that a regular hexameric individual dividing in complete non-directive endocoels will produce two symmetrical or two asymmetrical pieces. The number of symmetrical and asymmetrical pieces produced by such divisions should be approximately equal. Actually four divisions of this sort gave eight symmetrical pieces. The chances in such a division of a regularly octameric individual are two to one in favor of producing two asymmetrical pieces as against two symmetrical pieces. One such division gave two symmetrical parts. As far as these few cases have any signifi-

cance, they point to a tendency on the part of symmetrical individuals to divide into symmetrical parts by a vertical plane perpendicular to the directive plane.

Since a precisely symmetrical old piece has like mesenteries adjacent to its edges, whereas an asymmetrical piece may have like or unlike bounding mesenteries, any tendency toward the formation in division of accurately symmetrical pieces would be indicated by an excessive number of like bounding mesenteries. This gives us an opportunity to discover the limits of the tendency. The numbers of like and unlike bounding mesenteries of tables 3 to 6 are summarized in available form on the left of table 9. We may best disregard the less common classes and consider only the twenty-eight old pieces with two complete non-directive bounding mesenteries, thirty-two cases of one complete and one incomplete mesentery, and nine specimens with two incomplete bounding mesenteries. If we arbitrarily distribute into two equal groups the thirty-two cases of pieces with unlike bounding mesenteries, we have data suitable for the use of Yule's 'Coefficient of Association' ('00, p. 272) as a measure of a possible tendency of the division plane to pass through similar spaces on opposite sides of the directive plane. The distribution of the divisions in these cases is, then, as given in table 1.

Complete positive association, the invariable association of two incomplete or two complete mesenteries on the two boundaries of a piece, would be represented by a value for Q of + 1. Complete negative association, the constant association of a complete mesentery on one boundary with an incomplete mesentery on the other boundary of a single piece, would be indicated by a value for Q of − 1. Merely chance association would be indicated by a coefficient of 0. The calculated coefficient with its probable error is -0.008 ± 0.02. The coefficient, being less than its probable error, is practically zero. Our figures, therefore, give no indication of a departure from the chance association of two incomplete mesenteries, an incomplete and a complete one, or two complete mesenteries as old bounding mesenteries lying in one piece adjacent to a single division plane.

TABLE 1

	Complete	Incomplete
Complete	a = 28	b = 16
Incomplete	c = 16	d = 9

The coefficient of association by the formula,

$$Q = \frac{ad - bc}{ad + bc}, \text{ is } -0.008$$

The probable error of Q by the formula,

$$\text{P. E.} = 0.6745 \times \frac{1 - Q^2}{2\sqrt{a + b + c + d}} \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}}, \text{ is } \pm 0.02$$

This implies that the demonstrated tendency of the plane of fission to lie perpendicular to the major transverse axis of the mouth (p. 177) is not so strict as to regard the difference between two adjacent endocoels, the one complete, the other incomplete.

The same facts as were summarized in the left part of table 9 have been represented in the corresponding part of table 10, counting separately each bounding mesentery. The bounding mesenteries indicated by symbols in the column headed B in tables 3 to 6 are represented in table 10 in the column headed 'Frequency' and in the appropriate rows. Thus each unit in the frequency column of table 10 indicates a position of a division plane on one side of one individual resulting from a division, and the total number of units in the column of frequencies is equal to the number of symbols in all the columns of bounding mesenteries in tables 3 to 6. In reckoning the total number of divisions in complete and in incomplete endocoels I have subtracted the numbers of mesenteries whose apparent complete or incomplete character is in doubt. I have, of course, included

in the total number of incomplete endocoels those mesenteries undoubtedly incomplete but of undetermined grade. The percentages have been figured on the revised totals. The table shows division planes as follows: complete endocoels, 62 per cent; incomplete endocoels, 34 per cent; exocoels, 4 per cent.

The significance of these figures, in showing tendencies of the division plane to pass in spaces of a certain character, is affected by the relative numbers of spaces of each character in the animals at the time of division. Possibly the relative width of the spaces should also be taken into account. At an early stage in the formation of a cycle of incomplete mesenteries the adjacent exocoels are so reduced that the sum of the widths of endocoels considerably exceeds the sum of the exocoels. This condition gives way to a state of practical equality between endocoels and exocoels so soon that the temporary inequality probably has little if any influence on the relative frequency of passage of the plane of fission in different spaces. Certainly, the inequality between endocoels and exocoels is not sufficient to account for more than a small fraction of the excess of endocoelic divisions recorded.

The number of exocoels in an anemone is precisely equal to the sum of all endocoels. According to chance, neglecting inequalities in size, 50 per cent of the divisions should occur in exocoels. As shown in table 10, division planes occur in exocoels in only 4 per cent of cases. It is clear, therefore, that there is a strong tendency of the division plane to pass through endocoels.

The proportions here found do not agree with those given by Torrey and Mery for S. davisi. Their results, stated by the method I have used, give twenty-nine cases of division planes passing through exocoels to seventy-three passing through endocoels. These results, as Professor Torrey informs me, were obtained from hand sections of the anemones. Some incomplete mesenteries might be overlooked by that method. This might be responsible for the recording of a somewhat greater proportion of divisions in exocoels than I have found from the study of microtome sections, but it seems scarcely possible that it can account for the whole discrepancy. Even if the divisions in incom-

plete endocoels below the first grade are added to the exocoels as shown in my table 10, these form only 12 per cent of all the divisions, whereas Torrey and Mery found 28 per cent of all the positions of division planes to be in exocoels. Apparently, S. davisi and S. luciae differ in the proportion of divisions in exocoels.

As between incomplete and complete endocoels the chances cannot be accurately stated, since the cycles of incomplete mesenteries are variable in number. Probably division occurs very rarely if at all in a region where the first cycle of incomplete mesenteries is not yet established. The members of this cycle are equal in number to the complete mesenteries. Usually incomplete mesenteries of the second grade are also present. These are twice as numerous as those of the first cycle. Later cycles are sometimes present, at least in the older parts of a specimen. We may say, therefore, that probably not less than 50 per cent nor greatly more than 75 per cent of endocoels are incomplete; and that while, according to chance, divisions should occur in incomplete endocoels in at least as many cases as in complete endocoels, the actual numbers found are 57 incomplete to 103 complete endocoels, or 36 per cent of divisions in incomplete endocoels. There is, then, a marked tendency to divide in complete endocoels.

Since in regeneration the full number of complete mesenteries to be formed appears very promptly and the directive or non-directive characteristics are obvious at an early stage, the chances of a later division plane passing in directive or non-directive endocoels may be quite accurately determined. The old parts of animals represented in tables 3 to 6 show 572 complete mesenteries. Of these, 154 are directives. Therefore approximately 27 per cent of the complete endocoels of these animals are directive endocoels. Of 103 divisions in complete endocoels (as calculated for table 10), six, or approximately 6 per cent, are in directive endocoels. That is, of the divisions in complete endocoels there were less than one-fourth as many divisions in directive endocoels as chance would demand. This is only another expression of the fact, already demonstrated

(pp. 176, 177), that the plane of fission is more or less nearly perpendicular to the directive plane. That directive endocoels are avoided, gives additional emphasis to the tendency to divide in complete non-directive endocoels.

The inequality of the parts resulting from a division is striking. The relative size of the parts may be roughly measured by the number of complete mesenteries. Of fourteen divisions of diglyphic specimens into two parts each, no case of equal distribution of the complete mesenteries between the two parts is found, whereas cases of extreme inequality do occur. The other specimens show in general a similar lack of equality of parts. One might explain the inequality in the products of division of a regular hexameric individual on the ground that the tendency to divide in complete endocoels and the tendency to divide in a plane perpendicular to the major axis of the mouth overcome any tendency to divide into equal parts. But this does not hold for the unequal division of a regular octameric specimen such as no. 9 (table 3). In this specimen the tendencies for the fission plane to pass through complete endocoels and to pass perpendicular to the major mouth axis would both be satisfied by a division which should produce equal parts. Yet this division was very unequal. In a number of other cases the inequality of parts is much greater than is demanded by the tendencies to divide in complete endocoels and along the favored plane. It should be noticed that, in every case where a recent regeneration zone is indicated in the old part (tables 3 to 6), the piece containing this regenerating part possesses the greater number of complete mesenteries. It is possible that there is a tendency toward an equality in which different regions have values dependent upon age. The tendencies of the planes of fission so far as analyzed are summarized on pages 226, 227. It remains to be pointed out that, even among specimens having the same number of siphonoglyphs and complete mesenteries, the immediate products of division are quite diverse in form. The same processes, dealing with specimens already varying as shown in tables 3 to 7, lay a foundation for the most extreme numbers of mesenteries and siphonoglyphs found in the fully

regenerated individuals. Regenerative processes may begin with pieces varying in number of siphonoglyphs from none to three, and in number of complete mesenteries from zero to fifteen.

COURSE OF REGENERATION

The course of regeneration as seen in the living animal has been described above (p. 167 ff). The account which follows is derived from the study of sections. It should be noted that the white lines and orange stripes fade out immediately upon killing by any of the methods I have used. The external distinctions between new and old tissue are also lost in the process of fixation by some methods, but in formalin the newer area remains distinguishable by its lighter color, at least in those cases where the color differences in the living specimens are great. There is, nevertheless, little uncertainty as to the relation of external and internal features. After examining a series of stages in regeneration the boundaries between new and old material can be quite as accurately determined in sections as in living individuals, and the external markings and internal structures can in almost all cases be satisfactorily correlated.

In sections of specimens killed a few days after division the column wall in the region of fusion appears much like the old column wall. In some specimens this region is distinguishable on account of its being slightly thinner and having the outer border of the ectoderm less scalloped. This condition occasionally extends slightly into a part of the column which, as indicated by the mesenteries, is old. In the greater number of cases, practically no evidence of the region of fusion is found in the column wall a few days after division. In the esophagus evidence of the division persists much longer, especially at its aboral extremity. Closure of the esophagus orally occurs within four days. At this time its torn edges are contracted, the esophagus being much shorter on that side than on radii distant from the plane of division. Its full length on the regenerating side seems to be reached only as the new complete mesenteries become

attached to it. Because of this contraction and the consequent oblique position of the aboral aperture of the esophagus, the normal edge is often cut in transverse sections of regenerating individuals. The torn lateral edges of the esophagus before their union appear precisely like the normal free edge, and no clear boundary between these edges can be distinguished. Sections therefore show the esophagus open laterally, and it is impossibe to determine, in some cases, whether or not fusion has been completed. Certainly, the union proceeds down the column somewhat in advance of attachment to it of the developing complete mesenteries.

Formation of new siphonoglyphs

Following closure of the esophagus, a siphonoglyph appears along the line of fusion of its edges. A siphonoglyph differs from the remainder of the esophagus most obviously in its evenly rounded outline, the other portions showing in sections scallops with their convexities toward the cavity of the tube. A fully developed siphonoglyph shows also, in sections well preserved and stained, more abundant cilia than are to be seen in other regions and usually a more prominent heavy line marking the basal bodies of the cilia In consequence of these distinctive features, siphonoglyphs may usually be readily distinguished from other regions of the esophagus. The time at which differentiation of the siphonoglyph becomes clearly evident varies greatly, but is usually about ten days after separation. The undifferentiated esophagus continues to grow laterally on both sides of the new siphonoglyph, leaving the latter permanently in the middle of the regenerated region.

When division has occurred in the plane of a siphonoglyph, this structure persists and an additional siphonoglyph is formed. Three clear instances of this are recorded as nos. 21a, 21b and 44, the siphonoglyphs being indicated in tables 5 and 6 by the pairs of directive mesenteries with which they are invariably associated.

Recalling the types of division described above (p. 174 ff.), it is now clear how specimens with various numbers of siphonoglyphs may arise. Diglyphic specimens most frequently give rise to diglyphic forms, but may produce triglyphic (nos. 16b, 21a, 21b) or monoglyphic (nos. 16a, 21c) ones. Triglyphic individuals may produce diglyphic (no. 22a), monoglyphic, triglyphic, or tetraglyphic specimens. A tetraglyphic form (no. 15) did give rise to one individual with four siphonoglyphs, and one with two. Other possibilities are at once evident. It is wholly probable that any form may give rise, by one or two divisions, to any form. Since a new siphonoglyph is invariably formed in the new region at an early stage of regeneration, it is obviously more appropriate to refer specimens in process of regeneration to their ultimate class rather than to the one to which they appear at the moment to belong. It is therefore no longer admissible to say, as Davenport ('03, p. 141, line 20) has heretofore done, referring to the usual type of division of a diglyphic individual, that "a monoglyphic S. luciae is the result of longitudinal division of a diglyphic form," or to refer to the "metamorphosis of a monoglyphic to a diglyphic type" (ibid., line 33). The true monoglyphic type is a fixed type which does not develop a new siphonoglyph, thus transforming into a diglyphic type, although it may produce one or even two diglyphic individuals by a single division. The division of a diglyphic individual in the great majority of cases results in two diglyphic forms, never two truly monoglyphic ones.

Development of new mesenteries

By the time the siphonoglyph is clearly differentiated, all of the complete mesenteries to be formed may have appeared, but they do not reach the esophagus until somewhat later. New mesenteries show first in the lower half of the column. The earliest evidence of a mesentery is a very thin sheet of mesogloea extending from the mesogloea of the column wall inward into the endoderm. When the sheet has pushed nearly through the endoderm, the latter becomes projected inward in advance of it.

The new mesentery is then similar to the old incomplete mesenteries of the latest cycle except that the regenerated ones are usually thinner and have a much narrower and more lightly staining plate of mesogloea (for instance, figs. 7 and 8). They rapidly elongate vertically, extending downward to the base and upward to and across the oral disc; but the increase of a mesentery in thickness and in radial extent is slower until it has become complete by crossing the oral disc to the esophagus. It then rapidly extends centripetally, apparently as a result of tension, since a mesentery that has recently become complete is often thinner than one of the same age that has not quite reached the esophagus. At about the time a mesentery becomes complete in the extreme oral region, its longitudinal muscles become clearly evident (figs. 9 and 10). Subsequently these mesenteries grow thicker and their longitudinal muscles become more fully developed (fig. 13). They become attached to the esophagus farther and farther aborally. For a long period, however, at least some of the new complete mesenteries fail to reach the esophagus at lower levels where the old ones are attached (figs. 15 and 18: c^2, c^3).

The incomplete mesenteries arise in similar fashion, but they develop more slowly and retain much longer the slighter degree of development which distinguishes them from the incomplete mesenteries of the old region.

Number and arrangement of mesenteries in regenerated regions

Upon examination of the mesenteric formulas of the new regions of the specimens represented in tables 3 to 6, it is apparent that the variations in number and arrangement of the new mesenteries are determined almost wholly by the mesenteries on the boundaries of the old regions. Specimens listed in tables 7 and 8 give additional data upon which to base a study of regeneration formulas. Some of these were selected, in collecting, upon a basis which would interfere with their usefulness in considering the relative frequency of fission planes in different regions. Others were excluded from tables 3 to 6 on the ground

that, had the natural division occurred in some other plane, adequate evidence of the position of the plane of fission would not have persisted to the time of killing. Still others are regenerating individuals resulting from artificial cuts. The last mentioned are pointed out in the explanations of tables 7 and 8. It will be seen that they regenerate in the same general manner as if they had divided naturally in a corresponding plane, but apparently show wider variations in the number of new mesenteries.

Table 9 shows the frequency of different numbers of new complete mesenteries, based upon the specimens represented in tables 3 to 7. I include here as complete mesenteries not only those actually reaching the esophagus, but also mesenteries now incomplete but destined to become complete. These are, in nearly all cases, readily distinguishable from true incomplete mesenteries whatever the stage of regeneration. For incomplete mesenteries appear only after those destined to become complete have reached relatively large size, and they develop more slowly than the complete ones. Furthermore, the members of a pair of new incomplete mesenteries appear simultaneously and remain of equal size during their growth; while members of a pair of complete mesenteries reach such a state of equality only late in regeneration. Since all of the complete mesenteries destined to be formed in a regenerating region appear quite promptly, their number may be safely judged almost from the beginning. These features are illustrated in figures 23 to 30. The numbers of new mesenteries actually complete is indicated in the tables of mesenteric formulas in connection with each regenerating zone.

Where complete old non-directive mesenteries occupied both boundaries, regeneration involved the production of eight complete mesenteries in twenty-five cases and six complete mesenteries in two cases (table 9). The character and arrangement of the eight mesenteries commonly formed may be made clear with the aid of figure 15, representing specimen no. 64 of table 7. The older of the two regenerating sectors, occupying about two-fifths of the area of the cross-section (lower left), is limited by two old complete bounding mesenteries c, c. On either side of this regenerating region, adjacent to the old bounding mesentery, is

a new mesentery, c^2, forming with the bounding mesentery a non-directive pair. In the middle of the new area is a pair of directive mesenteries, d, d. Between the directive and the new bounding mesenteries, on either side, is a pair of non-directives, c^1, c^2. Figure 16, representing a more aboral section, shows the first cycle of incomplete mesenteries appearing in the primary exocoels. Other cycles of incomplete mesenteries are formed later. Where, under these conditions (as in specimen no. 26, table 6), six mesenteries are regenerated, only one complete non-directive is formed between the new directives and the old bounding mesentery, and this one mates with the latter. I have no specimen with the reduced number of complete mesenteries on both sides of the new directives, but no doubt such instances may be found. Only four new complete mesenteries would then be developed.

When division has occurred in two incomplete endocoels leaving an incomplete mesentery adjacent to each boundary of the old region, ten complete mesenteries are usually regenerated. This is the case in the later regenerating zone of the specimen (no. 81, table 7) shown in figures 15 and 16 (upper, right). In the middle of the new area is a pair of directives, *d*, *d*. On either side of this pair of directives are found two pairs of non-directives, c^1, c^2 and c^3, c^4. Permanently incomplete new bounding mesenteries, (*I*), (*I*) (fig. 16), are formed paring with the old incomplete bounding mesenteries. These new incomplete mesenteries appear later than the complete mesenteries, and in many specimens represented in the tables, they were lacking at the time of killing. In later stages of regeneration they are invariably found. They are frequently present in the aboral region while lacking at levels nearer the oral disc. This is the case in the specimen represented by figures 15 and 16. Other incomplete mesenteries develop in pairs in the exocoels of the new region. In three cases where both of the old bounding mesenteries are incomplete (nos. 4a, 4b of table 3 and no. 79 of table 7) only eight complete non-directive mesenteries were regenerated, and in one case (no. 76, table 7), but six. In these specimens only one pair of complete non-directive mesen-

teries was formed lateral to the directive pair on one or both sides.

When one of the bounding regions is occupied by a complete and the other by an incomplete mesentery, nine, seven, or five new complete mesenteries are formed. When nine are regenerated (as in no. 19a, table 5 and fig. 27), the new mesenteries consist of one pair of directives, a pair of non-directives on either side of these, a second pair of non-directives on the side toward the incomplete mesentery, and an additional complete mesentery pairing with the latter on the side toward the complete bounding mesentery. When but seven complete mesenteries are formed after division in one complete and one incomplete endocoel, the arrangement is the same as when nine are produced except that on the side of the incomplete mesentery two fewer complete non-directives are found. Sections, at two different levels, of a specimen (no. 66, table 6) regenerating in this way are shown in figures 12 and 13. Other specimens are represented in figures 11 and 19. In the case recorded as showing only five new mesenteries, two fewer are formed on each side.

Where a directive mesentery lies on the boundary, the new bounding mesentery is a directive mating with the old one, and two pairs of non-directives are formed on that side of the wholly new directive pair (for instance, nos. 21a and 21b, table 4). In one case (no. 10b, table 2) the new bounding mesentery adjacent to the old directive was not, at the time of killing, developed to a stage where it could be distinguished from an incomplete bounding mesentery.

On a side where an exocoel is involved in the division (for instance, nos. 54 and 84, table 5), regeneration is precisely the same as in a case where an incomplete mesentery occupies the boundary, except that no new bounding mesentery is formed. In none of the cases I have observed are there less than two pairs of non-directives on such a side. Two peculiar and interesting cases (nos. 18b and 22b, table 5) possibly illustrate the influence of the old bounding mesenteries on the regeneration, and suggests its limits. No. 22 was a specimen regenerating

from an experimental cut. Mesenteries of this regeneration are represented in table 5 by Roman type. The specimen divided into two parts, a and bc, in incomplete endocoels of the first grade, but with the loss of one of the bounding mesenteries in a. Eighteen days after this division, fission again occurred in bc, producing moieties b and c. On one side (the left in the table) this new fission plane passed again adjacent to the same old incomplete mesentery of first grade, which in this division passed to c. Meanwhile the normal number of mesenteries had been established, including the mate to this old bounding mesentery, now represented on the far right of the formula of b. The case of no. 18 is somewhat different. Here the division into a and bc was followed after but two days by the separation of bc into b and c. This occurred on one side along the boundary between old tissue and that just beginning to regenerate. The early state of this regeneration at the time of the second division may have had something to do with the irregularities indicated by the queries as to original completeness or incompleteness of two of the mesenteries. Certainly, in the two days that elapsed between the divisions, few mesenteries could have been established in the earlier regenerating zone, and none would give any indication of their final state of completeness if this had been determined. It appears that, while a regenerated normally, producing a mate to its incomplete bounding mesentery, the corresponding regenerating zone of b shows no corresponding incomplete bounding mesentery (which would be represented on the far right of the upper row of the formula for no. 18b). Rather, the two regenerating zones have matched up together, with two complete mesenteries from either side of the boundary forming a new pair. It must be noted that the uncertainties of identification in this case are such that great dependence must not be placed on the interpretation here suggested. Such a juxtaposition of two regenerating areas may account for the large number of mesenteries (including two pairs of directives) recorded in an apparently single regenerating region in specimens nos. 88 and 89 of table 8.

From these statements it appears that the chief variations in number and arrangement of mesenteries on each side of a regenerating area are wholly dependent upon the old bounding mesentery on that side, except for two complete non-directives, which may or may not be present whatever the character of the bounding mesentery.

Five specimens showing exceptional features in their regeneration are represented in table 8. Two of these specimens show an abnormally large number of new mesenteries,—one (no. 88) a second pair of directives, the other (no. 89) a second pair of directives and two additional pairs of non-directives. A third (no. 87) shows a new region which is normal except for the weak stage of development of the muscles in the pair of mesenteries in the position ordinarily occupied by the directives. These mesenteries extend to the esophagus, but their longitudinal muscles are so weakly developed that the directive or non-directive character of the pair cannot be determined. The fourth (no. 86) shows this pair of mesenteries extremely reduced. They do not reach the esophagus and show no evidence of longitudinal muscles. The fifth (no. 85) shows a reduction from the usual number of mesenteries, there being only one new bounding directive and two pairs of non-directives. An old regeneration zone in no. 40 (table 5) shows an excess of two mesenteries over the usual number.

Neglecting the few exceptions (six in all) just referred to, we may say that in regeneration a new pair of directives is formed, approximately in the center of the new zone, and that on each side of this pair of mesenteries are formed one or two pairs of complete mesenteries plus an odd mesentery if required to mate with a bounding mesentery on the edge of the old part. Treating separately each lateral half of a new zone, the tendency in full regeneration is to produce on each side of the middle plane a directive and three non-directive mesenteries; if a complete old bounding mesentery is not present, additional mesenteries are formed to mate with the odd new non-directive and with any old incomplete bounding mesentery.

TABLE 2

FORMULA	REGENERATED MESENTERIES	OLD BOUNDING MESENTERIES
No. 1 a	*d*, c^1c^2, c^3	c
No. 1 b	*d*, c^1	c
No. 2 a	d, c^1c^2, c^3c^4, (*1*)	(1)
No. 2 b	*d*, c^1c^2	(1)
No. 3	*d*, c^1c^2, c^3c^4, *d*	d
No. 4	*d*, c^1c^2, c^3c^4	

Table 2 gives, in a form favorable for reference, the normal regeneration formula of one side of the new area when the adjacent old bounding mesentery is a complete non-directive, **c**; an incomplete mesentery, **(1)**; a directive, **d**; or when no unpaired old bounding mesentery is present. The symbols used are those adopted for the formulas of tables 3 to 8 (see explanation, p. 232), except that the complete non-directive mesenteries are numbered with exponent figures. Mesenteries represented by the same symbol in different regenerating regions correspond, in completeness or incompleteness, in location with respect to the new directive plane, in position of the longitudinal muscle bands, and, as will appear later, in size relations from a short time subsequent to their first appearance until the new complete mesenteries reach a degree of development equal to that of the old mesenteries. It must not be assumed, however, that the complete non-directive mesenteries designated by the same exponent are in all cases homologous (cf. p. 197).

When less than the maximum number of mesenteries represented in the above formulas are regenerated, two non-directives on either or both sides of the new directives are commonly lacking. The missing mesenteries are probably those designated c^2 and c^3. The reduced number is found in approximately 50 per cent of all cases of regeneration following division in an endocoel of the first incomplete grade, but rarely following division in other planes. This is brought out in the right half of table 10.

It will be seen that the full number of mesenteries (4) in the half of a regenerating zone adjacent to a complete non-directive bounding mesentery is found in 93 cases, the reduced number (2) in 3 cases. On the side of old incomplete bounding mesenteries of the first grade the number of instances of the reduced number (3 mesenteries) is greater (22 to 21) than the number of cases of fuller regeneration (5 mesenteries). Toward incomplete bounding mesenteries of second or third grade, no reduced numbers are found; and the same is true of regeneration following divisions in exocoels. No reductions of the type here considered are recorded for regenerations after division in directive endocoels. The instance of regeneration of but five complete mesenteries under these conditions is no. 10b of table 3. This specimen shows an incomplete new bounding mesentery in place of the directive that would be expected to pair with the old bounding directive.

It may be well to call attention here, on the one hand, to the rather fixed character of mesenteries and other structures added in regeneration—a set of structures only slightly variable except as modifications near the edges are necessary to enable this set to fit into normal Hexactinian order with old parts adjacent to the boundary—and, on the other hand, to the highly variable result of such a regenerative process superimposed upon the process of fission previously described. The extremely variable number of complete mesenteries is thereby fully explained.

From what has been said of the number of mesenteries found in the new region, it is clear that the production of new mesenteries does not continue indefinitely. On the contrary, the result of the process is strictly limited. In the following pages it will be shown that the new mesenteries appear in a quite definite order.

Order of development of mesenteries

For my study of the order of development of mesenteries in regenerating regions, I have used such naturally divided specimens represented in tables 3 to 6 as showed sufficiently early stages of regeneration, and also some specimens that had been

artificially cut. Of the latter I have used only such as show clearly the distinction between new and old sectors and are not complicated by the presence of mesenteries extending through only part of the length of the column.

About three days after fission has become complete, two mesenteries appear approximately in the middle of the space between the two old bounding mesenteries. This space may appear much like an ordinary endocoel (fig. 4), but is usually somewhat wider. At this stage the two new mesenteries are sometimes (as in the specimen referred to) united by their inner edges forming a loop. Occasionally they retain this connection until they are complete orally and have well-developed longitudinal muscles; but usually they soon separate at the tips, as indicated in figure 5, which represents a more aboral section of the animal shown in figure 4. Very soon after this two other mesenteries appear between the first two. This establishes a set of four mesenteries, which retain the same relative size nearly up to the time when they become complete. This set of four is a striking feature of the regenerating zone for a considerable period even after other mesenteries have appeared. It probably corresponds with the group of four mesenteries found in S. davisi by Torrey and Mery ('04) and represented in figure 5 of their paper. These four mesenteries appear in their characteristic relations in the photographs shown in my figures 7 and 8.

The inner members of this set of four ordinarily become complete slightly in advance of the outermost ones. They form the directive mesenteries. The outer members of the set of four become complete and remain the nearest complete non-directives on either side of the new directive pair. They are the mesenteries referred to as c^1 in the account of the mesenteries of the new region, and are so labeled in the figures. The first four mesenteries of the new region are formed in the order described, no matter in what spaces division has occurred. The order of development of additional complete mesenteries, as well as their number, depends chiefly upon the old bounding mesenteries.

As shown above (p. 186), on the side of a regenerating region adjacent to a complete old bounding mesentery, two additional

mesenteries destined to become complete almost invariably appear. One, lying nearer the bounding mesentery and developing for some time slightly in advance of the other, becomes the mate of the old complete bounding mesentery. This is designated c^3 in the figures. The other complete mesentery becomes the mate of c^1. It is referred to as c^2. On the side of the new directive plane toward a complete old bounding mesentery the order of formation of the complete mesenteries is therefore as follows: c^1, *d*, c^3, c^2. This order is apparent, through differences in size of the mesenteries, in figures 9 and 10, representing sections at different levels of a single animal. The order in which the new mesenteries become complete is somewhat different from the order of their appearance, the directives usually being first to reach the esophagus, followed very shortly by c^1. c^2 very soon equals c^3 in size, and these two become connected with the esophagus at a somewhat later time. For a long time their inner ends are free from the esophagus near the aboral end of the latter. Occasionally the inner ends of c^2 and c^3 are united as described for the new directives. Instances of the stage where all of the new complete mesenteries except c^2 and c^3 are attached to the esophagus are represented in figs. 15, 18, and 31. As has been previously remarked by Carlgren ('04, p. 52), it was probably this stage in regeneration which the Hertwigs ('79, p. 82) took for a stage in ontogenetic development in the case of two specimens of Adamsia.

On the side of the first set of four toward an incomplete old bounding mesentery, the next mesentery to appear is likewise destined to become the outermost complete mesentery of the new piece. The longitudinal muscle, when it appears, faces toward the new directives, whereas the muscle of the outermost mesentery adjacent to an old complete mesentery faces (see above) toward the old part. This mesentery is c^4. The next to appear is the one designated c^2. It is destined to pair with c^1; c^3, the mate of c^4, appears very slightly after c^2, or even simultaneously with it. An incomplete mesentery, (*1*), appears adjacent to the incomplete bounding mesentery at a later period, as described below.

Whether the old bounding mesentery is complete or incomplete, soon after the appearance of the mesenteries destined to become complete and about the time these reach the esophagus, pairs of the first order of incomplete mesenteries appear in the exocoels between d and c^1 and between c^2 and c^3. This is shown in an early stage on the right sides of figure 13 and of figure 11, and in the older regenerating regions of figures 16 and 6. In some cases these pairs appear simultaneously, but commonly the pairs nearest the directives are slightly in advance of the pairs nearer the boundary. Considerably later, pairs of a second cycle of incomplete mesenteries appear in their characteristic positions, alternating with the pairs of both the complete and the incomplete mesenteries of the first order. See, for instance, figures 17, 27, and 28. In cases where the old bounding mesentery is an incomplete mesentery of the first grade, the new one (*I*) mating with it appears about the same time as the first cycle of incomplete mesenteries in the regenerating part, but in nearly all cases as the first of the mesenteries of this order. It remains the largest mesentery of its cycle for a considerable period. This mesentery is shown in figures 13, 11, 16, and in the older regenerating region of figure 6. When, on the other hand, the old incomplete bounding mesentery is of the second order, the new incomplete mesentery (*II*) pairing with it appears much later, at about the same time as the incomplete mesenteries of the second grade in other parts of the new region, usually as the first representative of this cycle. Two such new incomplete mesenteries may be seen in figure 17. An apparent exception is noted in connection with no. 80, table 7. It thus happens that one old and one regenerated mesentery, constituting a pair, may be definitely assigned to a certain cycle. Furthermore, the bounding mesentery is the only one of this cycle to be produced between the boundary and the nearest pair of new complete mesenteries, no incomplete mesenteries of higher grade being formed in this space. Consequently, after regeneration following division in an incomplete endocoel of the second order, the space including the boundary and lying between two adjacent pairs of complete mesenteries will lack incomplete mesenteries

of the first cycle unless a pair of these were included in the old part. As a result, normally divided and regenerated specimens occasionally lack a pair of incomplete mesenteries of the first cycle between two adjacent pairs of complete mesenteries. As will be shown later (p. 210), this involves the loss of an orange stripe, giving rise to an uneven number of these externally observable features. An unexplained lack of a pair of incomplete mesenteries of the first cycle between two pairs of new complete mesenteries is evident in the specimen represented in figure 6. Some instances have appeared which show two pairs of incomplete mesenteries of approximately equal size unseparated by mesenteries of a higher grade. I have among my sections perhaps half a dozen examples of this anomalous condition (p. 212). I have no explanation for it. Neglecting incomplete mesenteries except the single bounding one, the order of appearance of new mesenteries on the side of the new directive plane toward an old incomplete mesentery may be indicated as follows: c^1, *d*, c^4, c^2, c^3, (*1*).

Soon after c^1 becomes complete orally, c^4 becomes equal to it in development. c^2 and c^3, as in the case of division in a complete endocoel, become equal in size, but lag considerably behind their mates in becoming complete. Various stages in regeneration on this plan are represented in figure 6 (older regeneration), 11 and 13 (reduced regenerations), and 15.

I have few examples of early stages of regeneration where the old bounding mesentery is a directive. Two such cases indicate that the new bounding directive appears at a stage very slightly in advance of the paired incomplete mesenteries, i.e., at the same stage as an incomplete bounding mesentery. Examples of later stages show the bounding directive fully as well developed as the pair of directives in the middle of the new area. It is possible that the new bounding mesentery in such cases develops more rapidly in the intermediate stage about the time when it reaches the region of the oral disc and esophagus.

I have no clear cases showing early stages of development after division in exocoels. One would expect the order of development to be the same in such cases as when division is in

incomplete endocoels, except for absence of new 'bounding' mesenteries.

In the foregoing account, the determining influence of the old bounding mesenteries has prominently appeared. This influence appears early, and finally results in an adjustment of old and new parts that restores in the bounding region the normal pairing of mesenteries of a given cycle, and, usually but not invariably, the regular alternation of pairs of different cycles.

In connection with the mesenteric formulas of regenerated regions, a word of caution was given concerning assumptions of homology between mesenteries bearing the same designation. The reason for this may now be made clear. Mesenteries indicated by the same symbol in different formulas (table 2, p.191) are similar in character, as directives or non-directives, in position of the muscle banners (toward or away from the directive plane), and in location with respect to other mesenteries. If we retain these designations but place them in the order of their development, we have the following as the chief formulas:

No. 1a c^1, d, c^3, c^2
No. 1b c^1, d
No. 2a c^1, d, c^4, c^2, c^3, (*1*)
No. 2b c^1, d, c^2, (*1*)

Formula no. 1b differs from no. 1a in the absence of mesenteries c^3 and c^2. In formula no. 2b the third mesentery is designated c^2, but may really be homologous with c^4 of formula No. 2a. In that case the reduction here also consists in the suppression of c^2 and c^3. If this is correct, strict regard for homology would require that the mesenteries labeled c^2 in the lower part of figure 11 and on the left of figure 13 should be labeled c^4. In one or two instances I have found mesenteries c^2 and c^3 in a very early state of development when the other complete mesenteries were united with the esophagus through the greater part of its length. Whether these would have attained full development or would have disappeared cannot be determined. In either event these cases may represent a condition intermediate between the more complete development and the

reduced regeneration, and may point significantly to c^2 and c^3 as the mesenteries omitted in the reduced type of regeneration. Comparing formulas no. 1a and no. 2a as given above, we find that the third mesenteries to appear are different. Under these circumstances it is impossible to decide which mesenteries of the two formulas are truly homologous.

It will be noticed that, as described above, the members of a regenerating pair of complete mesenteries, except directives, do not appear simultaneously. Although attaining eventually to a condition of approximately radial symmetry, complete mesenteries arise in regeneration in bilateral fashion, a member on one side of the directive plane corresponding in degree of development with one in similar position on the opposite side of the directive plane. There is no reason to suppose that the same is not true of the development of mesenteries in the metamorphosis from the larval state. The complete mesenteries of S. luciæ, therefore, are all to be regarded as primary mesenteries, belonging to the first cycle of mesenteries, which is very generally found arising in a bilateral manner in the ontogenetic development of Hexactinians (cf. p. 207). Perfect bilateral symmetry of a regenerating region is often prevented through the influence of unlike old bounding mesenteries, or by unknown factors which cause the suppression, on one side of the directive plane, of mesenteries present on the other.

Results of repeated fission and regeneration

It has been shown that the products of fission in a group of specimens of S. luciae vary greatly in numbers of siphonoglyphs and of complete mesenteries. The former were found in specimens recorded in tables 3 to 7 up to three, the latter up to thirteen. It has been shown, further, that there is added in regeneration a set of structures including a siphonoglyph and a pair of directive mesenteries, together with other mesenteries including mates to the old bounding mesenteries. The complete mesenteries on either side of the new directives may vary from one to five, making possible a total addition in regeneration

of four to twelve. As a matter of fact, the smallest number added in any case of complete regeneration as recorded in these tables was five and the largest number eleven.

The average results of repeated divisions and regenerations may be derived from the data at hand. On the right of table 9 are given the numbers of mesenteries regenerated in zones of different types. The same data are given, considering separately each lateral half of a regenerating region, on the right of table 10. As explained in connection with the latter table, the average number of complete mesenteries in regenerating zones of all types is 8.4. It is obvious, therefore, that repeated divisions into two parts followed by regeneration will tend toward an average of approximately 17 complete mesenteries. Table 11 exhibits the number of complete mesenteries after regeneration for the specimens recorded in tables 3 to 6, and the average number of such mesenteries for each siphonoglyphic class and for the whole. The latter average is 15.8. The fact that this is below the number toward which repeated division and regeneration tend, points to a still lower number of mesenteries in the form resulting from ontogenetic development.

The average number of complete mesenteries, before division, of specimens represented in table 4 (including only those individuals all of whose fission products were available for record) is 13.5, a number considerably below the average for all the specimens of tables 3 to 6. In each of the cases given in table 4, the two closely succeeding divisions resulted in rapidly increasing the number of complete mesenteries. Although it may be purely a coincidence that apparently multiple divisions have, in these observed instances, occurred in specimens with a low number of complete mesenteries, it is possible that such divisions serve in an adaptive way to bring about a rapid increase. Whether this is correct or not, certainly the variations described for the processes of fission and regeneration are adequate to account for much wider variations in form of regenerated specimens than have been encountered. This indicates clearly the probability that there are correlations in these variations that are as yet unproved.

DISCUSSION OF REGENERATION IN HEXACTINIANS

The only previous detailed accounts of radial regeneration of sea-anemones comparable with the foregoing are those of Carlgren ('04 and '09) and Cary ('11). I shall now review the work of these writers in so far as it bears upon the problems here considered. Carlgren dealt with the regeneration of Sagartia viduata, Metridium dianthus, and Aiptasia diaphana. Of the last-named species only naturally produced basal fragments were studied. In Metridium, the material considered of natural fragments, artificial pieces of the same character as those separated naturally, and pieces cut from the base of the parent polyp in such a way as to exclude, so far as possible, all tissue of the column and of the mesenteries. I shall refer to these last as 'basal pieces.' Of Sagartia viduata, which does not reproduce naturally by asexual methods, artificial fragments of various forms and sizes were used, including some 'basal pieces.'

Natural fragmentation in Metridium and Aiptasia consists in the separation from the parent polyp of a small portion of the base and adjacent wall of the column with the adhering parts of mesenteries. The fragment thus receives only a very small proportion of the material of the parent polyp. The products of division in S. luciae, while they may be far from equal, are, so to speak, of the same order of magnitude, and each contains some part of the base, column, esophagus, circle of tentacles, and set of mesenteries.

Resorption of old mesenteries

In Metridium and S. viduata degeneration of mesenteries is evidently a prominent feature of the process of reconstruction. In these forms, however, degeneration does not commonly go to the extent of eliminating all of the old mesenteries. In Aiptasia, as stated by Andres ('82), rearrangement of mesenteries begins before the separation of the fragment from the parent. Carlgren believes that degeneration of the mesenteries, also, begins before separation is complete. He leaves in doubt the extent to which degeneration may go, since he was unable to determine

with certainty, in his sections, any boundary between old and new regions. His account is supplemented in this respect by that of Cary ('11), who studied the regeneration of three species of Aiptasia. According to Cary, all of the old mesenteries are resorbed, first at the oral extremity of the piece and progressively down the column until they have disappeared entirely.[3] Cary studied the process of regeneration following pedal laceration in another species (from Beaufort, North Carolina) that has been incorrectly known as Cylista leucolena. Presumably degeneration of the old mesenteries occurs in this species as in Aiptasia. Resorption of old mesenteries, with the possible exception of occasional members torn during fission (see p. 171), does not occur in S. luciae.

Sequence of new mesenteries

A brief account of the various types of arrangement and order of appearance of the mesenteries in regenerating anemones, as described by Carlgren and Cary, will be followed by a discussion of Carlgren's theories concerning the relation of these types to one another.

Figure 35 shows the types of arrangement of complete mesenteries found by Carlgren. Parts enclosed in the dotted lines indicate old regions, without an attempt to represent the number

[3] After examining Carlgren's original figures ('04, Taf. IX, Fig. 4 and 7) and text, I cannot agree with Cary ('11, p. 94) that "it seems very evident that all of the mesenteries shown in Carlgren's Fig. 7, Taf. IX, are old ones which have come over in the fragment from the parent individual and which will never come to be a part of the permanent system of mesenteries of the actinian arising from the laceration embryo." His interpretation of this figure may be correct, but the evidence for it is by no means conclusive. Criticism of another sort is due for Cary's treatment of Carlgren's paper in other respects. Glaring inaccuracies in interpreting the statements concerning the figures mentioned above are fortunately largely exposed by his quotation from the text. His mutilation of Carlgren's excellent figures is not so obvious to anyone not having the latter's paper at hand. Comparison with the originals of the figures (Cary, '11, pp. 92, 93) purporting to be copied from Carlgren's paper ('04, Taf. IX, Fig. 4, 7), reveals amazing discrepancies. To alter a figure in such fashion is a violation of the privilege of copying, even if modification be acknowledged; to do it without such admission is an offense against both the author, whose work is thereby misrepresented, and the reader, whose confidence is abused.

or character of the old mesenteries. Differences in size of the members of a pair of mesenteries in the diagrams of this figure indicate the order in which the mesenteries of the pair become complete. Table 12 sets forth the frequency with which these types occurred in regenerating pieces of different species. Cary's regenerating specimens of Aiptasia and Cylista (?) belong to type IV. The cases I have given of regeneration in S. luciae which do not involve matching up of new with old complete mesenteries are of Carlgren's types III, VI, or II. For convenience in indicating the order of appearance of these mesenteries, I have added to diagrams I and III the symbols by which I have designated mesenteries occupying corresponding positions with respect to the new directive plane. Using these symbols for the different mesenteries, we may construct formulas for the order of appearance as given by Carlgren in these different types, thus:

(I) c^3, c^1, d, c^2, c^4
(II) c^1, d, c^2.
(III) c^1, d, c^4, c^2, c^3.

Carlgren's statements indicate that in (I) c^2 and c^4 appear nearly simultaneously. In (III), c^4 appears only slightly after c^1, and c^3 very soon after c^2. It will be seen that the sequence of mesenteries in development given for type (III), as well as the order of becoming complete, is the same that I have found to occur in S. luciae except in cases where a complete old bounding mesentery is present.

Carlgren has proposed ('09, p. 41) an ingenious theory concerning the relations of the types of arrangement of mesenteries demonstrated in his regenerating specimens. For the arguments he produces in its support, the reader is referred to Carlgren's paper. I shall here simply outline the theory.

Types I, VII, IX, and X show clearly bilateral arrangements of the mesenteries in development. Type VIII is typically biradial in form. Type I Carlgren takes to be the most primitive. It parallels the Edwardsian method of ontogenetic development except in that the presence of the old part inhibits

the so-called ventral pair of directives. Type II might be considered as having been produced by reduction from either a bilateral or a biradial condition, but Carlgren regards it as a modification of Type I through the failure to develop of the innermost mesenteries (*d*) there represented. According to this view, then, *d* of Type I is lacking in Type II, and c^1 of Type I becomes *d* of Type II. Type IV is a combination of Types I and II, and Type X is the result of a doubling of Type I. Type VII represents an extreme expression of the tendency to the bilateral arrangement of mesenteries shown normally in Type I. Type IX represents normal development, such as Type I, in a basal piece having no part of the old column to interfere with the formation of the ventral directives. In explanation of Type III it is assumed that there are here two regenerating regions, each similar to that of Type II. In one of these regions the directive mesenteries are replaced by mesenteries of the old part. Type VIII is similar to Type III, but lacks old parts which might occupy the position of one pair of directives. Type V is a mixture of Types II and III; Type VI, of I and III.

It may be pointed out that the explanation for Type III is not entirely in harmony with the order of development of that type, but would demand the order c^1 and c^4, *d*, c^2 and c^3. It seems to me, however, that we cannot profitably consider at present the more abstract questions of the relation between the biradial and bilateral types of development. We cannot expect to solve these problems of form determination from examination of data collected for other purposes and assembled into tables and diagrams. The most that can be expected of such material from this standpoint is that it may present definite problems and suggest favorable points of attack.

One of these problems concerns in a concrete way the relations between the biradial and bilateral plans of regeneration. Carlgren has shown that both types may occur in the same species or even in the same regenerating pieces, and he has made a beginning in ascertaining the conditions determining the plan of development. He has shown that in Metridium larger pieces containing part of the base, column and mesenteries develop,

almost without exception, according to the biradial plan; but pieces containing material from the base alone frequently develop on a bilateral plan. In S. viduata the same influence of the character of the material in the regenerating piece is found with a greater tendency, whatever the nature of the piece, toward a bilateral plan of development. It may be that the rounding up of the more homogeneous material of basal pieces results in conditions similar to those influencing the development of mesenteries in ontogeny.

Siphonoglyphs and directive mesenteries

We cannot go far in considering the relation between these different types of mesenteric development without being confronted with the more general question of the determination of form in ontogeny and in regeneration. Carlgren's discussion of the relation of Types I and II suggests one of these, i.e., the governing influence of the siphonoglyph. He puts forward ('09, p. 43) the idea that, upon the formation of the siphonoglyph during regeneration, this structure immediately determines that the pair of mesenteries latest formed in the same plane shall be directives, and that no other mesenteries shall be formed in this plane. According to this view, the difference between Types I and II is due to the earlier stage at which the new siphonoglyph is established in II. The latest bilateral pair of mesenteries formed at the time of the appearance of the siphonoglyph becomes the pair of directive mesenteries, and no new bilateral pairs arise except those mating with mesenteries already present to form radially placed non-directive pairs.

My observations lend some support to this hypothesis in so far as it involves the determination of the directives by the siphonoglyph; but I believe that in S. luciae delay in formation of a siphonoglyph does not lead to the production of more than two bilateral pairs of mesenteries adjacent to the potential directive plane. As I have already indicated, the siphonoglyph appears shortly after closure of the wound in the region of the mouth. At about this time some of the new mesenteries reach

the esophagus. Previously the longitudinal muscles of the new mesenteries are not apparent, but about the time these mesenteries reach the esophagus the muscles rapidly develop and the directives are distinguishable from the non-directives. The order of events suggests that the development of mesenteries into directives is determined by the presence of a siphonoglyph. In a number of cases I have found a group of four small mesenteries with the characteristic proportions of the first four regularly formed in regeneration, but extending only a short distance up and down the column. Elsewhere the column wall and mesenteries gave no evidence of a division which might have given occasion for such a regenerating area. I interpret these as regions of regeneration following comparatively slight injuries to the body wall. In most of the instances the mesenteries are small and have no indication of longitudinal muscles. In no case do mesenteries in these sets show the characteristics of a pair of directives. In one case apparently the two inner mesenteries of the four have the characteristics of non-directives. In another instance two mesenteries only are formed. These are long and slender and show no longitudinal muscle bands. They reach the esophagus in a region where histological evidence of a siphonoglyph is not present, although there was a slight groove at that region of the mouth, as seen from the exterior and in sections, and although a narrow white line, indicative of the presence of a siphonoglyph, was to be seen in the living animal extending part of the way from the groove toward the tentacular zone of the disc.

These observations suggest that the first four mesenteries which form so constant a feature of the regeneration of S. luciae arise independently of the siphonoglyph, and that contrary to Carlgren's hypothesis, no more mesenteries are formed adjacent to the directive plane even in the absence of a siphonoglyph. The order of events in S. luciae is as follows: fusion of edges of the column, appearance of a set of four new mesenteries, extension of these to the esophagus, formation of a siphonoglyph, development of longitudinal muscles in positions which mark out the inner members of the first set of four mesenteries as a

pair of directives. This order of events may be interpreted as an epigenetic form-determining series, in which event A leads to event B, etc.

Influence of old bounding mesenteries

A most striking difference between the regeneration I have described for S. luciae and that of all the types given by Carlgren and Cary consists in the total absence from the latter of any variation ascribed to the influence of old mesenteries. In Aiptasia, where old mesenteries are resorbed, and even in cases of Types IV and IX in S. viduata, where little or no old tissue belonging to mesenteries or column is present, the lack of influence of old mesenteries upon regeneration is not surprising. But in many other cases, in both S. viduata and in Metridium, well developed mesenteries apparently exert no influence over regeneration. The variations in arrangement of new mesenteries in the species described by Carlgren are of wholly different character from the variations seen in S. luciae, which are governed almost completely by the mesenteries on the torn edges of the old piece. The influence of these bounding mesenteries in S. luciae is apparent in the earliest stages of regeneration. They have no obvious effect upon the directive mesenteries nor upon the first non-directive mesenteries, which precede the directives. The character of the bounding mesentery may, however, determine the nature of the third mesentery on either side. If the bounding mesentery is complete, this third mesentery becomes its mate and a fourth mesentery, which becomes the mate of the first non-directive, very soon appears. If, on the other hand, the bounding mesentery is incomplete, the third mesentery is followed by a fourth and a fifth. The determination of the number of mesenteries is effected before these mesenteries have their longitudinal muscles developed. My impression, here as in connection with the determination of siphonoglyphs and directives, is that we have to do not with one, but with a number of form-determining influences successively brought to bear. Some of these influences have been suggested, but we are far

from having any complete list of the factors concerned, and from understanding fully their order of effectiveness, much less their fundamental nature.

Pairing of mesenteries

The tendency to form, ultimately, unilateral pairs of mesenteries, i.e., pairs whose members lie on the same side of the directive plane, is apparently very strong (except in the case of directives) in the regenerating regions of all these species of anemones. This is especially evident in S. luciae, where it usually involves the matching up of regenerating mesenteries with old ones. There is no evidence, however, that non-directive mesenteries destined to become complete ever arise in regeneration as unilateral pairs. In every case described, one member of the pair precedes in development. According to their manner of development, then, the complete mesenteries are what have been commonly referred to as 'primary' mesenteries. They correspond in order of appearance with the 'Hauptsepten' of the Hertwigs ('79, pp. 81, 88), with the 'protocnemes' of Duerden ('02, p. 388), and with both the 'protocnemes' and the 'deuterocneme's of McMurrich ('10, p. 4). The first cycle of mesenteries arising as unilateral pairs in the primary exocoels are, then, secondary mesenteries. They are 'metacnemes' according to Duerden's terminology and 'zygocnemes' in McMurrich's. As already stated, there is no evidence that the latter ever become complete in the forms whose regeneration has been studied.

ORANGE STRIPES AND CINCLIDES

Some observations on the relation of the orange stripes to the processes of fission and regeneration in S. luciae have been made by Davenport ('03, pp. 140, 143). Her statements will be referred to in connection with the evidence I have collected bearing upon the points involved.

In a well-expanded living animal with brightly colored orange stripes it is not difficult to determine the relative positions of

siphonoglyphs, directive mesenteries, and orange stripes. An orange stripe is invariably found opposite the siphonoglyph between the lines of attachment of the members of the pair of directive mesenteries. As Davenport has stated, the orange stripes occur only in endocoels. Comparison of the number and position of the orange stripes as seen in the living animal, with the number and position of the mesenteries as found in sections, indicates that the orange stripes lie between the members of pairs of complete mesenteries, and also, contrary to the statement of Davenport, of incomplete mesenteries of the highest grade. For instance, the specimen a section of which is represented by figure 17 showed ten orange stripes; those represented by figures 19, 21, 22, and 26 showed twelve stripes each. In a regenerating specimen in which all the mesenteries and stripes of the new region are formed, the above statement holds (with rare exceptions to be mentioned later) for both new and old parts.

The frequency with which division occurs in the endocoels occupied by orange stripes suggests the possibility that these stripes have some functional significance in the processes of fission or regeneration. It is clear, however, that the presence of such stripes is not essential to the normal progress of these processes. Specimens divide spontaneously in other regions, and the resulting pieces, as well as similar fragments artificially produced, regenerate readily. Furthermore, the related species, S. davisi, reproduces freely by the same method, yet is destitute of any such stripes.

Davenport ('03, p. 143) calls attention to the fact that the cinclides occur on the stripes. I find, however, that they are not confined to the orange stripes. Figures 33 and 34 show three cinclides, clearly marked by protruding acontia, none of which are in positions occupied by orange stripes. In figure 19 at x appears another cinclis, likewise not situated on an orange stripe. Under favorable conditions of lighting, the cinclides are readily visible on the living specimen with the aid of a hand lens, and their distribution may be accurately determined. They are found in vertical rows in all positions in which fission planes may

pass. That these openings affect the position of the division plane is possible but quite unlikely. If they are of significance in the location of the fission plane, their distribution should show some relation to the frequency of fission in different planes. As to this, no evidence is at hand.

The fact that cinclides are commonly located in other positions as well as on the orange stripes is evidence against the idea suggested by Davenport ('03, p. 143) that the presence of the stripes may "be considered as a case of warning coloration?"

Orange stripes in fission and regeneration

During the process of fission, an orange stripe lying in the endocoel cut by the plane of division has been observed, in a few cases, to be divided by the tearing of the column wall. After the division the narrow border of orange along the edges of the pieces must be promptly absorbed, for in no case observed has there been found early in regeneration an orange stripe on the boundary between the old tissue and the new. On the contrary, nine specimens in early stages of regeneration which were killed and sectioned after counting the orange stripes give clear evidence of lack of stripes in positions almost certainly containing them before the division. At the time these were killed none showed any orange stripes in the newest regenerating area. Of the nine individuals, six represent the paired products of three divisions (nos. 11, 13 and 14, table 3) and three are unpaired specimens (no. 30, table 6, a specimen represented in figure 9, and one other). All of the paired and two of the unpaired specimens showed division through two complete endocoels. The third unpaired specimen had divided through endocoels of the first incomplete grade. All divided in planes where orange stripes were to be expected. Furthermore, the paired specimens showed orange stripes in such numbers and positions as to occupy all regions, outside of the new area, in which they normally occur, indicating that the orange stripes were probably fully formed before the division occurred. In none of these cases was there any evidence of an orange stripe marking the

endocoel on the boundary between new and old parts. In all of them the number of observed orange stripes corresponds with the number of complete and first order of incomplete endocoels lying wholly within the old part. Thus the specimen of which a section is shown in figure 9 had three orange stripes in the positions indicated diagrammatically in figure 36. The other unpaired specimens showed five and seven orange stripes, respectively, corresponding with the number of undisturbed endocoels normally bearing stripes. Each of the pairs had three orange stripes in one member and seven in the other similarly situated. The indication given by these nine specimens is that an orange stripe lying in a space cut by a plane of fission is lost.

In regeneration following division through an endocoel occupied by an orange stripe, a new stripe is finally developed in the bounding endocoel in addition to those formed in endocoels lying entirely within the new region. Were this not true, complete endocoels lacking orange stripes would be common, whereas they are actually exceedingly rarely, if ever, found. The formation of new bounding mesenteries, when division occurs in one of the lower grades of incomplete endocoels, has already been described (p. 195), the result being that the mesenteries of a pair enclosing the boundary between new and old regions are of the same grade. The bounding endocoel consequently may be definitely designated as belonging to a certain cycle. When this bounding endocoel is of the second incomplete grade or of a lower order, no orange stripe is formed in it. The endocoel on the boundary between new and old regions, is, then, no exception to the general rule that orange stripes are formed in endocoels of the complete mesenteries of the first cycle, but not of lower cycles. In support of this statement I may cite a number of examples. Anemone no. 82, table 7, a section of which is shown as figure 17, had divided through incomplete endocoels of the second grade or possibly lower. This specimen, when killed, had ten equidistant orange stripes, equaling the number of endocoels of the first two cycles. Evidently there were no orange stripes in the bounding endocoels.

Specimen no. 80, table 7, gives similar evidence. The number of orange stripes and their position in relation to the new area as observed before killing are shown in figure 37. It was noted that possibly one of the orange stripes, here represented as lying within the new area near one boundary, might be an old stripe. Study of sections shows in the old part a pair of directive mesenteries and an adjacent pair of non-directives of the first incomplete grade, the plane of fission passing through secondary incomplete endocoels lying lateral to these. The two old and nine new orange stripes correspond in position with the complete endocoels and the incomplete endocoels of the first cycle. Evidently orange stripes were not produced in the secondary incomplete endocoels occupying the boundary. As a result of this position of the division plane, on one side there is a space between two pairs of complete mesenteries that does not contain a pair of incomplete mesenteries of the highest grade. In all probability there was no orange stripe in this region—two adjacent orange stripes occupying complete endocoels. This illustrated the seldom realized possibility of the normal production of a fully regenerated specimen with an odd number of stripes, one less than twice the number of pairs of complete mesenteries. Another specimen showed exactly the same arrangement of complete mesenteries, incomplete mesenteries of the first order, and orange stripes, but with the position of the bounding planes no longer evident. Another specimen with twenty-one orange stripes and eleven pairs of complete mesenteries is similarly explained. Sections of this individual give evidence of two regenerating regions, both in a very late stage. Probably three of the four division planes involved passed through complete endocoels; one evidently cut an incomplete endocoel of the second order. Both old and new mesenteries bounding the latter endocoel are clearly of the second order. This is the only region bounded by pairs of complete mesenteries in which representatives of the first cycle of incomplete mesenteries are lacking. Undoubtedly this accounts for the lack of one orange stripe from the number usually found in a specimen with eleven pairs of complete mesenteries. Anemone

no. 54, table 6, showed ten orange stripes and (internally) six pairs of complete mesenteries. On one side the boundary between old and new evidently lies in an incomplete endocoel of the second or lower grade. This accounts for the lack of one orange stripe between two adjacent pairs of complete mesenteries. On the other side an undisturbed old incomplete endocoel of apparently the first order is present, and an orange stripe would be expected there. In the absence of an orange stripe in this region, one is driven to consider the possibility of this pair's belonging really to the second order of incomplete mesenteries rather than to the first cycle of which it is apparently a member. In that case four cycles of incomplete mesenteries must have been present. If this is so, division must have occurred in an exocoel. Both the presence of four cycles of incomplete mesenteries and divisions in exocoels are uncommon occurrences, and this combination is consequently improbable and the explanation unsatisfactory.

In the specimens just described, orange stripes were not found on the boundary between old and new regions when this lies between members of the second cycle of incomplete mesenteries. If this always held true and the incomplete mesenteries were invariably regularly placed, we should never find orange stripes associated with two pairs of incomplete mesenteries not separated by a pair of complete mesenteries. It has been shown previously (p. 196.) that irregularities in the first cycle of incomplete mesenteries are occasionally found. Thus two pairs of incomplete mesenteries of the largest size with no complete mesenteries separating them were found in three specimens on which counts of orange stripes gave odd numbers. Regeneration was apparently complete in these cases, and the number of stripes is such as to lead one to suppose that in each case one occupies the additional incomplete endocoel. In these instances a reduction from the typical number of orange stripes, which is double the number of pairs of complete mesenteries, or an excess over the typical number, corresponds with a departure from the normal number of incomplete mesenteries of the first order.

For the sake of completeness, two specimens showing still wider variations from the typical conditions should be described. One of these on which ten orange stripes were counted shows mesenteries that would account for but eight. The other two stripes may have been situated in a broad expanse of the body wall between two pairs of complete mesenteries, in which are seen one pair of large incomplete mesenteries and an irregular lot of small ones, of doubtful history. They vary in number and in size at different levels. I have no explanation to offer for the presence of this group of mesenteries or of the stripes associated with them. The other specimen showed a single stripe in its older part and none in the newer region. Sections revealed five pairs of complete mesenteries and three complete mesenteries whose mates were not yet complete. A specimen with mesenteries present in such numbers and degrees of completeness would ordinarily show at least seven orange stripes.

In the preceding paragraphs are described all of the specimens I have observed and sectioned which constitute exceptions to the general statement that the number of orange stripes in an undivided or fully regenerated specimen is twice the number of pairs of complete mesenteries. Of the nine such exceptions, three, showing one less than the typical number of stripes, are explained on the ground of division in an incomplete endocoel of second or lower grade resulting in elimination of one endocoel normally occupied by an orange stripe; three with an extra orange stripe show, each, an additional unexplained pair of incomplete mesenteries of apparently the first order in whose endocoel the supernumerary stripe probably lay; while three show wholly unexplained deviations from the normal relations of stripes and mesenteries. Over against these exceptions must be urged the significance of forty-nine specimens on which the number of orange stripes as determined before killing was exactly double the number of pairs of complete mesenteries as ascertained by examination of sections. Undoubtedly in these cases the orange stripes occupied the complete endocoels and incomplete endocoels of the first order only. These forty-nine specimens show

great diversity in features other than the relation of stripes to mesenteries. Thus two have eight stripes; eight have ten stripes; twenty-three have twelve stripes; five have fourteen stripes; nine have sixteen stripes; one has eighteen stripes, and one has twenty stripes. Also thirty-six are diglyphic; twelve are monoglyphic, and one is triglyphic. Four, of which one is represented in figure 26, show no evidence of having undergone division, while the others give more or less evidence of regeneration, and a few show clearly the precise position of the plane of fission. Of the latter, some represent divisions in complete endocoels, some in incomplete endocoels of the first grade, and one, of which a section is represented in figure 17, in incomplete endocoels of the second order. Most of the specimens are biradially symmetrical, but a number depart from this condition. The triglyphic specimens and diglyphic individuals with an uneven number of pairs of complete mesenteries cannot be strictly biradially symmetrical. It is clear, therefore, that in nearly all cases undivided specimens, or those in advanced stages of regeneration, have orange stripes corresponding in number and position with the complete endocoels and the highest order of incomplete endocoels.

The number of incomplete endocoels of the first grade being almost invariably equal to the number of complete endocoels, the number of orange stripes is twice the number of pairs of complete mesenteries in the overwhelming majority of cases. Nevertheless, in any given population of this species one finds a large proportion of specimens showing an odd number of orange stripes. These are to be accounted for, in small part, on the basis of the irregularities in arrangement of the incomplete mesenteries described above (p. 211). Thus the exclusion of a pair of incomplete mesenteries of the first cycle from a primary exocoel owing to a division in an incomplete endocoel of second or lower grade adjacent to a pair of complete mesenteries, would reduce by one the number of orange stripes in the fully regenerated individual. Again, the unexplained duplication of incomplete mesenteries of apparently first grade was shown to be associated with an extra orange stripe. By far the greatest

number of specimens showing odd numbers of stripes are specimens which have recently divided and have either developed no new orange stripes or have produced, at the time of observation, less than the full set to be acquired.

The number of the new orange stripes gives some idea as to the completeness of the regeneration. From what has been said of the number of mesenteries regenerated and of the relation of orange stripes to mesenteries, it is evident that the number of new orange stripes may vary from five to eleven. In case of division in one or two directive endocoels, twelve or thirteen are possible numbers. Numbers below seven or above eleven are, however, distinctly uncommon for single completely regenerated areas. Below seven it is likely that the stripes of the new region are not fully formed. Above eleven—unless one of the orange stripes on the boundary between new and old lines is opposite a white line on the oral disc and therefore occupies a directive endocoel—it is almost certain that the new area consists in fact of two regenerating zones of not very different age.

The number of mesenteries regenerated is such that repeated fission and regenerations (p. 198) wou d tend toward an average condition with about seventeen complete mesenteries. Since the complete mesenteries are always paired and correspond, with rare exceptions, with the orange stripes, we may say that the tendency is toward approximately eight pairs of complete mesenteries and sixteen orange stripes. Division followed by complete regeneration would rarely give rise to individuals with fewer than seven orange stripes, and never with fewer than five. The great majority of individuals with small numbers of orange stripes have not completely regenerated, while even among specimens with high numbers of stripes many have not yet completed the regeneration.

As to the order in which orange stripes appear in the new region, I have little information. Frequently the presence of stripes may be ascertained while as yet they are so faint in color and so close together that the number cannot be determined. At a somewhat later stage there is some reason to believe that stripes are present in complete endocoels when as

yet none can be seen between the members of pairs of incomplete mesenteries. One of my specimens indicates this. It was described, while living, as a triglyphic individual with three old orange stripes and eleven on the boundaries, or within, the new area (fig. 41). Sections show normal positions for the three old stripes. The sections also demonstrate that the regenerated area is composed of an older and a newer part. The three division planes are all in complete endocoels. Including the bounding endocoels there are in the new tissue eight complete endocoels. In the older regenerated part there are three pairs of incomplete mesenteries, all of the first grade, no mesenteries of a lower grade being present anywhere in the regenerated portion. In the most recently formed part there are four pairs of incomplete mesenteries. The relative position as well as the number of the orange stripes makes it probable that the eleven new orange stripes occupied all of the complete endocoels and the incomplete endocoels of the original piece and of the older regenerated area only. It is probable that new orange stripes would later have appeared in the incomplete endocoels of the first grade of the newest region. Another regenerating specimen showed externally ten orange stripes, whereas sections revealed six pairs of complete mesenteries and six pairs of incomplete mesenteries of the highest grade. It is probable that two of the second grade of incomplete endocoels in the new region lacked orange stripes at the time of observation. I have no clue as to which of the endocoels lacked the stripes.

The formation of orange stripes first in the complete and later in the first grade of incomplete endocoels would correspond with the order of development of the mesenteries of the regenerating region. This order of development of the stripes is in harmony with the statement of Davenport ('03, p. 143, and fig. 2) that new orange stripes appear between old ones. It cannot be too positively stated, however, that this formation of new stripes, as well as the production of new complete mesenteries, is confined to regenerating regions, and that the process is strictly a determinate one. After the formation of the group of complete mesenteries heretofore described (pp. 185 to 192) and of the first

cycle of incomplete mesenteries alternating therewith the number of orange stripes is absolutely limited and their positions are determined. The only occasion thereafter for the formation of new complete mesenteries and new orange stripes is a division initiating a new regeneration.

EXTERNAL INDICATIONS OF INTERNAL STRUCTURES

Much information concerning internal structures may be obtained by a consideration of all the external features of individual anemones. Differences in ground color of the column wall or in the width or intensity of color of the orange stripes persisting in spite of changes in the state of expansion of the specimen, especially if associated with unevenness in length, breadth, or whiteness of the white bars, give indication that fission has occurred and that regeneration is in progress. These features may, especially in the earlier stages of regeneration, present differences sufficiently sharply marked to indicate the precise boundary between old and regenerating tissue. An obstacle of no little importance is the liability of unequal contraction in different parts of the wall of the column to alter for the moment the intensity of all colors and the relative positions of the stripes. The intensity of coloration of the new as compared with the old part, the width of the new sector, and the presence or absence in it of a new white bar or of new orange stripes, give basis for judging within broad limits the stage of regeneration of internal parts. Frequently the presence of three or more sectors of different ages may be readily determined, and the different sectors may show various stages of regeneration. When two regenerating areas of nearly the same age lie adjoining each other, it is often difficult to recognize them as two. In certain cases it is practically impossible to distinguish by external observation the constituent parts of such a double regeneration. Whenever the number of orange stripes of an apparently single regenerating region exceeds eleven, it is almost certain that two regenerations are involved. When the orange stripes are faint or when they are less than seven in number in a given regenerating

sector, there is ground for suspicion that not all of the orange stripes of the zone have appeared, or that a part of the regenerating region has been separated off in a later fission. As previously stated (p. 167), there is danger, especially in faintly colored specimens, of counting as an orange stripe the narrow space representing the union of the torn edges shortly following a division. This is especially likely in faintly colored individuals. It should be constantly guarded against when counting stripes. When no slightly developed regenerating area is present the internal structure may in most cases be accurately inferred from the number of orange stripes. Thus Davenport's ('03) figure 11 represents probably a specimen possessing material of four different ages. The oldest shows three orange stripes; the next oldest, two; the third, six, and the most recently formed area, nine. All except the last formed sector have been partially removed in the divisions which initiated the later regenerations. If, in addition, the number and position of white bars on the oral disc were known (thereby locating the directives), still other details might be surmised. Under favorable conditions even the incomplete mesenteries of second and later cycles may be identified through the column wall after complete mesenteries have been located. It should be clear, then, that careful examination of living specimens of S. luciae enables one to distinguish recently divided specimens and to estimate approximately the stage reached in the regenerating region; and, in specimens that have not recently undergone fission, to infer quite accurately and in detail the number, character, and positions of the mesenteries.

COMPOSITION OF NATURAL GROUPS

We may now look into the composition of colonies of this species and consider to what extent this composition is affected, or accounted for, by the processes of asexual reproduction described. This examination should also yield information concerning the sexually produced form of the species. With these questions in view, I have studied natural groups of individuals taken at different seasons from a variety of situations around Woods Hole.

Siphonoglyphs

The condition of these groups as regards number of siphonoglyphs is represented in table 13. These are referred to as 'unselected' specimens, but the conditions under which the collecting was done and the counts made allow of a considerable degree of selection in addition to the normal errors of random sampling. Each lot consists of a portion of a natural group of individuals collected at one time from a closely restricted locality, such as a single stone or a few stones of similar quality lying near together. Each lot was either taken immediately into the laboratory and examined or, when collected during the winter, taken to Cambridge and studied there. In order to determine the number of siphonoglyphs it was, of course, necessary to wait until the animals expanded, exposing the oral disc. Naturally, also, many of the specimens were expanded at one time and the removal of a few whose condition was noted caused many others to contract, necessitating much delay in completing the count. There was opportunity for unconscious selection because many were open at a time and because some show their condition clearly at a glance while others require close examination to reveal the state of their siphonoglyphs. In a few cases sections were necessary to determine the number of siphonoglyphs. Experience shows that external examination is not wholly reliable for this purpose and that, in case of any irregularities in the mouth region (cf. fig. 1, 4), only sections can give certain information. In each lot some specimens persistently failed to expand; some left the stones and were lost; and some underwent division probably as a result, in part, of the change in environment. The errors from all of these sources together undoubtedly render this table useless for any precise statistical analysis. Furthermore, the great variation among different lots indicates that, even if the accuracy of the numbers shown could be depended upon, the total numbers are much too small. Nevertheless, some idea of the relative magnitude of diglyphic, monoglyphic, and other classes may be obtained from the numbers given. As shown in the table, these groups gave

1101 diglyphic, 112 monoglyphic, 61 triglyphic, and 4 tetraglyphic individuals. It will be understood that all stages of regeneration were represented in specimens of these lots, and that consequently many specimens in early stages of regeneration failed to show siphonoglyphs that nevertheless would certainly develop in the middle of the new region (p. 183). Such potential siphonoglyphs were, of course, counted just as if they were actually completed.

Mesenteries

No attempt has been made to determine directly the number of mesenteries in numbers of individuals. Remembering the relation shown to exist between mesenteries and orange stripes, the counts of stripes presented hereafter have some significance from this standpoint. Consideration has been given, however, to the number of mesenteries of apparently undivided diglyphic specimens. Of the 1101 diglyphic individuals in the groups represented in table 13, 63 failed to show in the living state satisfactory signs of division. Forty-one of these were sectioned and studied for internal evidences of division. Among these, thirty gave unmistakable evidence of the sort already described (pp. 169, 170), five showed slight irregularities such as are commonly associated with fission and regeneration, while six were forms without any irregularities to indicate unlike ages of different parts. Of the six last mentioned specimens, five were biradially symmetrical with six pairs of complete mesenteries, and one had eight pairs. One with six pairs of complete mesenteries is represented in figure 26. Of the five slightly irregular and therefore possibly undivided specimens, two possessed six pairs of complete mesenteries, two had eight pairs, and one showed ten, all being strictly biradially symmetrical. These numbers are small, and their interpretation must be modified by the fact that, among fully regenerated forms, regularly hexameric individuals with twelve orange stripes (and by inference six pairs of complete mesenteries) are more numerous than other forms. It can, at most, be said that, among apparently undivided specimens, a regular form with six pairs of complete mesenteries is the most common type.

Orange stripes

The factors concerned in the determination of the number of stripes apparent on a given individual at a particular time are such that any mere enumeration of orange stripes in a set including recently divided individuals is of highly questionable significance. Nevertheless, a study of a tabulation of counts of orange stripes may yield suggestive results and may be made an occasion for pointing out further the effect of processes of asexual reproduction upon the external appearance of specimens.

Counts of orange stripes made upon four lots of specimens collected at Woods Hole are given in table 14, and the totals obtained by adding the first three are plotted in figure 42. In figure 42 are given, also, data published by Davenport ('03). If we look at the solid line in figure 42, representing a summary of my counts of July and September (lots 1, 2 and 3), we see that, beginning with twelve, the higher even numbers of stripes are represented by more individuals than the odd numbers. This is in harmony with the greater number of completed regenerations in the classes with higher numbers of stripes. The odd numbers here probably represent chiefly uncompleted regeneration. Below twelve, the odd numbers of orange stripes are most abundant. This is particularly true below eight. Among those lower numbers, recently divided specimens form probably the greater proportion of individuals. The predominance of odd numbers among these classes is in all likelihood chiefly due to those specimens which have formed no new stripes. With the relative positions of orange stripes and mesenteries of different orders in mind, it is obvious that divisions in two complete endocoels or two incomplete endocoels of the first grade would give usually odd numbers of old stripes; divisions in one complete endocoel and one incomplete endocoel of the highest grade would ordinarily give even numbers of old stripes; while divisions in other planes might give either even or odd numbers. The relative frequency of divisions in different planes (table 9) is such as to make the expected ratio of odd to even numbers of old orange stripes approximately 4 : 3. It is possible that during the

progress of regeneration of the stripes there is a tendency further to increase temporarily the proportions of instances of odd numbers.

Comparison of the data for the separate lots is rather suggestive. Lots 1 and 2, collected July 12 and 18, respectively, differ in that the earlier lot includes a slightly larger proportion of specimens with a very low number of stripes indicating repeated, rapidly succeeding divisions, while the later set shows a few more individuals with high numbers of stripes indicating more nearly completed regenerations. Lot 3, representing a group collected September 22 from the same place as lot 2, shows a relatively much greater number of individuals with twelve or more stripes. In this group the mode is at twelve with prominent secondary modes at fourteen and sixteen and only lower modes at seven and five. This indicates progress in regeneration with less frequent divisions since the collection in July. Lot *X* represents a group of specimens selected for their large size. The distribution as shown in the table suggests that the group is composed of specimens that have, for the most part, completely regenerated. Examinations of my records, which show for each individual the number of stripes in areas of all different ages, confirms this suggestion. Davenport's curve apparently represents a group of individuals of which a very large proportion have recently divided. It includes a few specimens with more than twelve orange stripes and a large number with twelve, but the great bulk of individuals show fewer than ten. Davenport gives no indication of the time at which these specimens were collected. The large number of individuals with few orange stripes, in connection with my curves, suggests the probability that they were collected early in the summer. Among the groups below ten Davenport's curve in contrast with mine shows an excess of specimens with even numbers of stripes. A possible partial explanation of this lies in the readiness (p.167) with which the line of fusion of cut edges soon after division may be mistaken for an orange stripe. One stripe thus added in a certain proportion of cases would alter the relative numbers of specimens with odd and even numbers of stripes from the state

shown in my groups to that given by Davenport's series. The large number of recently divided specimens in the latter's series would make this error possible in a large number of cases. The cases figured by Davenport ('03, figs. 3 to 7) are evidently in too early a stage of regeneration to permit of any difficulty on this point; but it would be encountered in dealing with slightly later stages. If we assume twelve to be the number of stripes typical of specimens resulting from ontogenetic development (see p. 225 for discussion of this point), the large number of specimens with twelve stripes as compared with those having higher numbers suggests the likelihood that there were included in Davenport's collection an unusual proportion of undivided specimens. In this connection the length of time the species has inhabited the region concerned may be significant. Possibly the entrance of S. luciae into a region and its establishment there is accomplished by migration of larval forms, while the propagation of the species in a region already occupied is much more largely brought about by the asexual method. That asexual reproduction has been in progress for a shorter time in the group studied by Davenport than in my lots is indicated by the relatively small number of specimens with more than twelve stripes, since I have shown that repeated regenerations tend toward the production of an average condition with about seventeen stripes.

FORM RESULTING FROM ONTOGENETIC DEVELOPMENT

From what has been said it is evident that no form as regards number of siphonoglyphs, mesenteries, or orange stripes certainly distinguishes regenerated individuals from those that have not undergone fission. The development of transforming embryos must actually be followed to get unquestionable evidence as to the form resulting from that process. I have made a rather careful search of certain restricted localities at all seasons of the year for such specimens with little success. The dates of my special searches at Woods Hole were January 21, 1905; July 12 and 18 and September 22, 1909; November 28, 1910;

March 31, April 30, May 30, and June 29, 1911. I have had opportunities at various other times for less careful observations of localities in which this species occurs. The only recently transformed anemone I have found is a very small and nearly colorless specimen collected at Woods Hole August 4, 1909. This specimen showed no trace of the green color or orange stripes of the column, or of the white line on the oral disc characteristic of S. luciae. Whether it belongs to this species, to Cylista leucolena, to Metridium marginatum, or to still another of the species of anemones found at Woods Hole, cannot be stated. In the absence of color it resembles C. leucolena, but we have no reason to believe that at this stage either S. luciae or M. marginatum have developed their characteristic colors. Whatever its proper classification, this anemone was symmetrical and diglyphic and had nearly attained a regular hexameric condition of the mesenteries. Each pair of complete non-directive mesenteries, however, had one member incomplete—that member lying in all cases toward the same end of the chief transverse axis. It is therefore in a stage intermediate between the Edwardsia condition and the regularly hexameric form. Even were it determined that this specimen represents a stage in the development of S. luciae, it is by no means certain that the attainment of the condition with six pairs of complete mesenteries would mark the end of its ontogenetic development. This specimen therefore throws no direct light upon the present problem. It does, however, suggest possible directions in which evidence may be found, and the circumstances connected with it indicate some of the difficulties in the way of a complete solution. The apparent scarcity of developing embryos emphasizes the impression that the extraordinary method of asexual reproduction is the chief and highly successful means of perpetuating this species and increasing its numbers.

In Hexactinians six is the most common fundamental number of pairs of complete mesenteries, as two is the typical number of siphonoglyphs. I have shown (p. 220) that such a form is the commonest one among apparently undivided specimens of S. luciae. I have shown further (p. 198) that while successive

divisions and regenerations in this species tend toward a condition with an average of approximately eight pairs of complete mesenteries and sixteen orange stripes, the actual averages in the populations studied are less than eight and sixteen. This is presumptive evidence that the form resulting from ontogenetic development possesses fewer than eight pairs of complete mesenteries and fewer than sixteen orange stripes. It appears from the statements of Davenport ('03, p. 143 and fig. 1) that twelve is a common number of pairs of complete mesenteries. This is clearly an error. Among several hundred sectioned specimens I have found but two with twelve pairs. These are both triglyphic individuals and both show clear evidence of having divided. I have not seen a single biradially symmetrical specimen with twelve pairs of complete mesenteries. Such a specimen would normally have twenty-four orange stripes, whereas the highest number recorded by Davenport or myself is twenty-two. It seems likely that Davenport mistook incomplete mesenteries of the first order for complete ones. This is the more probable since she indicates that the specimens described as having twelve pairs of complete mesenteries have twelve orange stripes, the number which I have shown to be characteristic of a form with but six pairs of complete mesenteries. Considering all the evidence at hand, it is probable that the sexually produced form is a diglyphic one with six pairs of complete mesenteries, six pairs of incomplete mesenteries of the first order alternating with these, and twelve orange stripes occupying these two sets of endocoels. Specimens of this form may also be produced by regeneration following fission, and all normal departures from this typical form are due to asexual reproductive processes.

SUMMARY

Sagartia luciae is typically Hexactinian in form and structure except for the wide variation in number of siphonoglyphs and of pairs of mesenteries. The number of siphonoglyphs and of associated pairs of directive mesenteries varies from one to five. The number of pairs of complete mesenteries including the

directives varies from five to twelve. Three or four grades of incomplete mesenteries may be found (p. 164 and tables 11 and 13).

Asexual reproduction in this species occurs by a process of aboral-oral fission with subsequent regeneration (p. 167). In this process movements of parts of the basal disc in opposite directions are initiated, centering in two or more isolated regions. These movements place the intervening tissues of the base under strain and result in a rupture of the basal wall. The rent progresses until base, column and esophagus are successively involved, and complete separation of the individual into two or more pieces finally results.

Details are recorded of the divisions of one monoglyphic, one tetraglyphic, three triglyphic, and seventeen diglyphic specimens (p. 174 and tables 3 to 5).

The resulting pieces may possess one or more siphonoglyphs and associated pairs of directive mesenteries or they may lack these structures until regeneration has occurred (pp. 174, 175).

Succeeding divisions may be delayed until regeneration is completed or they may occur at any earlier time. They may even follow so rapidly as to give the appearance of simultaneous division into more than two pieces. In every such case a regeneration zone is formed in each piece for each successive fission (p. 173).

In passing upward the plane of fission rarely cuts a mesentery, i.e., the plane is a strictly vertical one (p. 171).

There is no tendency toward strict equality of the products of a division (p. 181).

Strictly diradially symmetrical polyps tend to divide into parts which are themselves symmetrical with respect to the original directive plane, i.e., the plane of division tends to be approximately perpendicular to the major axis of the mouth (p. 176), but no tendency to divide in spaces of the same kind on opposite sides of the column was detected (p. 177).

While division may occur in any vertical plane, it tends to occur in endocoels rather than exocoels (p. 179), in complete endocoels rather than incomplete endocoels (p. 180), and in non-directive rather than directive complete endocoels (p. 180).

No obvious change occurs in the old part in consequence of division, except that possibly mesenteries injured in the process of fission are eliminated by absorption (pp. 168, 171, 172, 200).

Regeneration processes begin with the rolling in and fusion of the torn edges of the body wall (pp. 167, 182). In the region of fusion new structures are gradually differentiated, eventually constituting a large proportion of the bulk of the individual—often far the greater part (pp. 167, 182).

The torn edges of the esophagus also grow together and a new siphonoglyph invariably becomes differentiated in the region of fusion of these edges. As regeneration proceeds, the new siphonoglyph occupies the middle of the new region, thereby marking this as a new directive plane (p. 183). In those instances where a siphonoglyph is cut by the fission plane (p. 183), a siphonoglyph occupies the corresponding boundary between old and new parts of the regenerated animal and a wholly regenerated siphonoglyph is formed in addition. Origin of specimens with different numbers of siphonoglyphs from any of the common types is completely explained by the manner of division and regeneration (p. 183).

Four new mesenteries, constituting a very characteristic group, become established in the middle of the new region of the column and grow across the oral disc to the esophagus (p. 193). Longitudinal muscle swellings appear on each of the first four mesenteries on the side away from the directive plane (p. 194). Additional mesenteries follow in a bilaterally paired manner lateral to the first set of four (pp. 193, 198, 207). Among these later mesenteries certain members mate with the two outer ones of the set of four, forming unilateral pairs. The two inner members of this set of four constitute a pair of directive mesenteries. Other of the later mesenteries become paired with the old bounding mesenteries or with each other, so that eventually all of the mesenteries, with the exception of the directives, are present in the unilateral pairs characteristic of the Hexactinians (pp. 193, 207).

Variations in the number of mesenteries formed depend almost wholly upon the character of the old bounding mesenteries. On

the side of a new directive plane which is toward an old incomplete bounding mesentery are usually produced two pairs of complete non-directive mesenteries. On the side of the new directive plane which is toward an old complete non-directive bounding mesentery are commonly formed a pair of complete non-directive mesenteries and a single complete mesentery which forms a non-directive pair with the old bounding mesentery (pp. 186, 188). The only common exception to the numbers of complete mesenteries as stated consists in the omission of a pair of complete mesenteries lateral to the new directives (pp. 187, 188, 191). The number of complete mesenteries formed in a regenerating zone is thus strictly limited and almost invariable, except through the influence of the old mesentery adjacent to the boundary between old and new tissue. This mesentery, if unpaired, exerts a perfectly definite determining influence upon the course of regeneration, an influence which makes its appearance soon after regeneration has begun and effects a normal pairing of the mesenteries of the bounding region and usually a regular arrangement of the pairs of different cycles in harmony with those of other regions (p. 193).

The order in which the new mesenteries appear and the order in which they become attached to the esophagus (pp. 193 to 188, 201; see especially pp. 193, 196) do not correspond, and neither agrees with the order of ontogenetic development described for any Actinian whose transformation has been completely followed. At a late period of regeneration a stage is passed through corresponding with that described as a stage in the ontogeny of Adamsia by Hertwig; but this is probably correctly interpreted by Carlgren as a stage in regeneration (p. 194).

In the course of regeneration incomplete mesenteries appear in pairs in the normal positions (p. 194). Where the old bounding mesentery is an incomplete one a single new incomplete mesentery of the same cycle is formed to pair with it. When division has occurred in an incomplete endocoel of the second or lower order close to a pair of complete mesenteries, no new incomplete mesenteries of higher cycle are formed in the region limited by the complete mesenteries and including the boundary between

the old and new parts. Consequently a pair of incomplete mesenteries of the first grade and its accompanying orange stripes are sometimes lacking in a region where they ordinarily occur (p. 195). Certain other irregularities in the occurrence of incomplete mesenteries are found (p. 196).

The orange stripes seen prominently on the living specimens normally occupy the complete endocoels and the incomplete endocoels of the first order (p. 207). Since spaces of these grades almost invariably alternate regularly, the orange stripes are commonly present in even numbers in individuals that have not divided or have completely regenerated following a division (pp. 114, 122).

When a fission plane passes through an orange stripe, that stripe disappears (p. 209). As regeneration proceeds orange stripes are formed in their characteristic positions, including the bounding endocoels, provided these are not incomplete endocoels of the second or lower grade (p. 209).

Probably orange stripes arise, in the new area, first in the complete endocoels and only later in the incomplete endocoels of the highest grade (p. 215). The number of orange stripes is strictly limited by the number of mesenteries of the first two cycles, no new stripes being formed either in the old tissue or in the new tissue after the characteristic spaces are occupied (p. 216).

Odd numbers of orange stripes are usually to be explained on the basis of incomplete regeneration, occasionally on the ground of irregularity in the cycle of mesenteries of the highest incomplete grade Certain cases of unusual conditions of orange stripes remain unexplained (pp. 195, 210, 213, 221, 222).

The number, position, breadth, and color of the orange stripes of any individual give significant indications of its internal condition (pp. 215 to 217), but mere enumerations of orange stripes are of little value. The numbers of stripes in individuals of groups examined by the writer varies from zero to twenty-two, the plotted curve showing modes at seven and twelve with a tendency toward minor modes at even numbers above twelve stripes and at odd numbers below ten. This is interpreted as

an indication that among individuals with higher numbers of stripes a preponderance of specimens have completely regenerated; while among the lower numbers a great proportion have recently divided and regeneration of stripes has not begun or has at least not been completed. Such counts as have been made further indicate with some degree of probability that divisions are more frequent in the spring and less frequent toward fall (p. 222ff.).

By the processes of regeneration described, a rather definitely fixed set of structures is added to a piece resulting from division, regardless of the form of that piece (pp. 185 to 192). The outermost mesenteries of this set are modified to match up with the old bounding mesenteries. Since the forms of old pieces are quite various (p. 181), the resulting individuals are likewise diverse in the number of siphonoglyphs, mesenteries and orange stripes (pp. 184, 192). With rarest exceptions the variations from the typical Hexactinian form may be accounted for on the basis of the processes of asexual reproduction described. Repeated fission and regeneration tends to produce specimens with approximately eight pairs of complete mesenteries and sixteen orange stripes (pp. 198, 215). The fact that actual counts of mesenteries in specimens of the groups studied show a distinctly lower average than this, indicates that the sexually derived form possesses fewer than eight pairs of complete mesenteries.

The fundamental form of the species, i.e., that resulting from ontogenetic development, has not been ascertained. It is probably biradially symmetrical and hexameric, with two siphonoglyphs (pp. 218 to 220) and six pairs of complete mesenteries (pp. 220, 221), two pairs of which are directives.

Sexually mature individuals of both sexes have been found, but the development of the fertilized egg has not been followed. The failure to obtain developmental stages, together with the abundance of stages in asexual reproduction (p. 220ff.), suggests the probability that the latter constitutes the chief means of maintaining or rapidly increasing the population of a given region.

College of William and Mary, August, 1917.

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SYMBOLS USED IN TABLES AND FIGURES

The following symbols appear as subordinate headings under Old and New Mesenteries in tables 3 to 8:

B, bounding mesentery, i.e., a mesentery adjacent to the boundary of the new or old region in which it lies and without a mate in that region. The mesentery occupying this region is represented whether incomplete or complete.

D, directive mesenteries, which occur in pairs.

N-D, complete mesenteries which are not bounding mesenteries nor directives. With rarest exceptions they are found in pairs.

The symbols below are used in table and figures to designate specific mesenteries.

In bold face they represent mesenteries formed during ontogenetic development or in the oldest regenerating region distinguishable in that specimen.

In italic type they indicate mesenteries regenerated after the recorded division.

In roman type they represent mesenteries of intermediate age. Some of these may have been incompletely developed at the time of the latest division.

d, a directive mesentery.

c, a non-directive mesentery, actually complete or destined to become so.

c^1, c^2, c^3, c^4, regenerated non-directive mesenteries, actually or potentially complete, whose positions with respect to the new directive plane are indicated in the figures by exponent numerals.

c?, a mesentery now complete, but possibly derived from an incomplete one through abnormal fusion with the esophagus. It is consequently doubtful whether this was complete when fission took place.

(I), a member of the most advanced cycle of incomplete mesenteries represented in an individual.

(II), (III), members of the second and third cycles of incomplete mesenteries found in the specimen.

(1), a permanently incomplete mesentery of undetermined grade.

(1)?, an apparently incomplete mesentery which was probably derived from a complete one by being torn from the esophagus.

* symbol indicating absence of any unpaired mesentery adjacent to the boundary between old and new parts, i.e., the boundary lies in an exocoel.

† symbol used in table 5, no. 18b and no. 22b (and in table 10 referring to the same specimens) to designate the bounding conditions when a second fission plane has passed through the boundary between old tissue and tissue regenerating following a shortly preceding division.

Four, five, etc. (tables 3, 5, 6), number of small mesenteries in a regenerating region at a stage of development too early to indicate the final formula.

GENERAL EXPLANATION OF TABLES 3 TO 8

Mesenteric formulas of regenerated polyps are here represented in tabular form, but in a manner which has to some extent the significance of a diagram. In the first column of each table are given numbers designating different individuals or groups of related individuals. Letters in this column refer to different regenerating animals derived from a single anemone by fission. In the columns included under 'old mesenteries' are indicated the complete mesenteries and incomplete bounding mesenteries of the old part which persist without obvious change. Similarly, under 'new mesenteries' are represented mesenteries of the region formed after division. In all cases where regeneration is sufficiently advanced to give conclusive evidence of the final formula of the regenerated region, this is given in detail, except that definitively incomplete mesenteries are represented in the formulas only when they occur as bounding mesenteries. In cases of earlier stages only the number of new mesenteries present is indicated. The formula of each individual at the time it was killed is given between two adjacent horizontal lines. With the exception of a few complicated cases, specially explained in connection with table 5, the arrangement of complete mesenteries in any specimen whose mesenteric formula is given in detail can be readily pictured if, reading from left to right, one imagines the indicated mesenteries distributed in pairs around the body of the anemone with the last of the new mesenteries adjacent to the first of the old ones. Thus figure 9 shows a cross-section of a specimen having the same arrangement of complete mesenteries (disregarding the distinction between old and recently regenerated parts) as represented in the formula of no. 7b (table 3) after regeneration. Similarly, figure 18 may serve to illustrate the arrangement of complete mesenteries of no. 7a after regeneration, and figures 19 and 21 of no. 7 *before division*.

In the following three columns are given respectively the number of mesenteries in the regenerated region actually reaching the esophagus at the time of killing, the number of days during which regeneration had proceeded, and the numbers of the illustrative figures. Specimens represented in tables 3 to 6 may be fairly regarded as constituting a small random sample of the specimens found near Woods Hole, Massachusetts (pp. 170, 171). Those represented in tables 7 and 8 may not be so considered, but are useful as showing further instances of regeneration following division of the types they represent.

TABLE 3

In this table are given the mesenteric formulas of fourteen pairs of polyps resulting from natural fission and regeneration. For explanation of symbols, see page 232; for interpretation of the formulas, page 233. For a discussion of certain irregularities shown in table 3 to 5, see pages 171 and 172.

NO.	OLD MESENTERIES					NEW MESENTERIES					REGENERATION		FIGURES
	B	N-D	D	N-D	B	B	N-D	D	N-D	B	Complete	Days	
1 a	c	cc	dd	cc,cc	(II)	*four*					0	20	
1 b	c	cc,cc	dd	cc,cc	(II)	*six*					0	20	6, 7
2 a	(I)	cc,cc	dd	cc,cc	(I)	*four*					0	9	
2 b	(I)	cc	dd	cc,cc	(I)	*four*					0	14	
3 a	(I)	cc,cc	dd	cc,cc	(I)	*twelve very small*					0	10	8
3 b	(I)	cc,cc	dd		c	*four very small*					o	10	
4 a	(II)		dd		(I)	*(I)*	*cc*	*dd*	*cc,cc*	*(II)*	3	51	
4 b	(II)	cc,cc	dd	cc,cc	(I)	*(1)*	*cc*	*dd*	*cc,cc*	*(1)*	3	51	
5 a	(I)	cc,cc,cc	dd	cc,cc	c	*c*	*cc*	*dd*	*cc*		6	50	
5 b	(I)	cc,cc	dd	cc	c	*c*	*cc*	*dd*	*cc,cc*	*(I)*	9	50	
6 a	(II)		dd		c	*c*	*cc*	*dd*	*cc,cc*	*(II)*	5	49	
6 b	(II)	cc,cc	dd	cc	c	*c*	*cc*	*dd*	*cc,cc*	*(II)*	6	49	
7 a	c	cc	dd	cc	c	*c*	*cc*	*dd*	*cc*	*c*	8	45	
7 b	c		dd		c	*c*	*cc*	*dd*	*cc*	*c*	8	45	
8 a	(I)	cc,cc	dd	cc	c	*c*		*dd*	*cc*	*(I)*	5	56	
8 b	(I)	cc	dd		c	*c*	*cc*	*dd*	*cc*	*(I)*	7	56	11
9 a	c		dd		c	*four very small*					0	24	
9 b	c	cc,cc	dd	cc,cc	(1)?	*six small*					0	24	
10 a	(1)	cc,cc	dd		c	*c*	*cc*	*dd*	*cc,cc*		5	34	
10 b	d	cc,cc	dd	cc	c	*c*	*cc*	*dd*	*cc,cc*	*(I)*	3	34	
11 a	(1)?		dd		c	*c*	*cc*	*dd*	*cc,cc*	*(I)*	8	26	
11 b	c	cc	dd	cc	c	*c*	*cc*	*dd*	*cc*	*c*	5	26	
12 a	(I)	cc	dd	cc	c	*seven small*					4	20	
12 b	(I)	cc,cc	dd	cc,cc	c	*six very small*					0	20	
13 a	c		dd		c	*eight small*					0	20	
13 b	c	cc	dd	cc	c	*eight small*					0	20	
14 a	c	cc	dd	cc	c	*two*					0	7—	
14 b	c		dd		c	*two*					0	7—	

TABLE 4

In this table are shown the mesenteric formulas of two additional natural pairs. No. 15 showed some evidence of a preceding regeneration, but the limits of the regenerated region were not clear.

NO.	OLD MESENTERIES							NEW MESENTERIES						REGENERATION	
	B	N-D	D	N-D	D	N-D	D	B	B	N-D	D	N-D	B	Complete	Days
15 a...	c	cc	dd	cc	dd	cc,cc	dd	(I)		*cc,cc*	*dd*	*cc*	*c*	7	50
15 b...	c	cc,cc	dd	cc,cc				*		*cc,cc*	*dd*	*cc*	*c*	5	50
16 a...	*	cc,cc						(III)		*cc,cc*	*dd*	*cc,cc*		6	22+
16 b...	*	cc,cc	dd	cc,cc	dd			(III)		*cc,cc*	*dd*	*cc,cc*		6	22+

TABLE 5

This table presents the mesenteric formulas of six groups of polyps, each group derived from a single individual by natural fission. The number of zones of regeneration indicates that the multiple divisions involved are successive rather than simultaneous.

Certain regenerated polyps show two new areas. In these cases only bounding mesenteries are represented in the old region on the same horizontal line with the new mesenteries. Two such specimens (nos. 17, 19) have a pair of complete mesenteries in the old region between the two division planes. This pair is represented in the table in a horizontal space interposed between the lines showing the bounding mesenteries. To arrive at the complete formula of a polyp with two regenerating regions, the symbols of the first new area must be read, in reverse order, between the extremes of the old area represented on the same line. Similarly, the second new area must be inserted in reverse order between the limits of the old region shown on the same line with it. This may be most conveniently illustrated by no. 19b, of which a section is represented in figure 32. No. 19a is represented in figure 27, no. 19d in figure 30. The mesenteric formulas (disregarding incomplete mesenteries and distinctions between old and new mesenteries) of this and the other available fully regenerated products of division of no. 19 may be represented as follows:

No. 19a dd, cc, cc, dd, cc, cc (fig. 27).
No. 19b dd, cc, cc, cc, cc, cc, dd, cc, cc, cc, cc (fig. 32).
No. 19d dd, cc, cc, dd, cc, cc (fig. 30).

No. 19 was described before division as having two white bars on the oral disc and twelve orange stripes. Its formula was probably, like that of the specimens shown in figure 25, as follows: dd, cc, cc, dd, cc. cc. The lost part was very probably like No. 19c in having no old siphonoglyph or directives. No. 20 was triglyphic before fission, as determined by the white bars. The lost part must have possessed two old siphonoglyphs and pairs of directives.

Daggers (†) serve to call attention to boundaries in two cases, Nos. 18b and 22b, where apparently a second plane of fission passed in the plane of junction of old tissue with new tissue regenerating after a shortly preceding divison. For discussion see page 188.

TABLE 5—*Continued*

NO.	OLD MESENTERIES					NEW MESENTERIES					REGENERATION		FIGURE
	B	N-D	D	N-D	B	B	N-D	D	N-D	B	Complete	Days	
17 a...	(I)		dd	cc	c	*c*	*cc*	*dd*	*cc,cc*		2	13	
17 b	(I)			cc	c	*c*	*cc*	*dd*	*cc,cc*	*(I)*	4	18	
17 b	c				c	*four*					0	6	
17 c...	c	cc,cc,cc	dd		c	*four*					0	6	
18 a...	(I)	cc	dd		c	*c*	*cc*	*dd*	*cc*	*(I)*	7	42	
18 b	†				c?	*(I)*	*cc*	*dd*	*cc*	*c*	4	42	
18 b					c	*c*	*cc*	*dd*	*cc*	*c*	4	40	
18 c...	(I)?				c	*several very small*					0	40	
19 a...	c		dd		(I)	*(I)*	*cc,cc*	*dd*	*cc*	*c*	9	34–37	27, 28
19 b	c	cc			(I)	*(I)*	*cc,cc*	*dd*	*cc*	*c*	9	34–37	31, 32
19 b	(III)				c	*c*	*cc*	*dd*	*cc,cc*	*(II)*	5	34–37	
19 c...	Lost												
19 d...	(I)		dd		(III)	*(II)*	*cc,cc*	*dd*	*cc cc*	*(I)*	10	34–37	29, 30
20 a...	(I)	cc,cc	dd		c	*c*	*cc*	*dd*	*cc,cc*	*(I)*	9	58	
20 b	(I)				c	*c*	*cc*	*dd*	*cc*	*(I)*	6	58	
20 b	c				c	*c*	*cc*	*dd*		*c*	6	58	
20 c...	Lost												
21 a...	c		dd	cc,cc	d	*d*	*cc,cc*	*dd*	*cc*	*c*	10	59	
21 b	c				d	*d*	*cc,cc*	*dd*	*cc*	*c*	10	59	
21 b	c				(I)	*(I)*	*cc,cc*	*dd*	*cc*	*c*	9	59	
21 c...	c				(I)	*(I)*	*cc,cc*	*dd*	*cc*	*c*	9	59	
22 a...	(I)		dd		*		*cc,cc*	*dd*	*cc cc*	*(I)*	10	22+	
22 b	†				(I)	*(I)*	*cc,cc*	*dd*	*cc,cc*	*(I)*	10	22+	
22 b					d	*four*					0	4	
22 c...	(I)	cc,cc	dd	cc,cc	d	*four*					0	4	

TABLE 6

This table gives the mesenteric formulas of thirty-two unpaired polyps resulting from natural division and regeneration. In these specimens new complete mesenteries are distinguishable from old complete ones by their relative degree of development alone. All specimens in which new complete mesenteries could be distinguished from old only by their position with respect to an obvious boundary have been excluded from this table. This exclusion is necessary in selecting a group of animals that may be used for a study of the frequency of divisions in different spaces, since evidence of the position of the plane of fission is lost more quickly in complete endocoels and in exocoels than in incomplete endocoels. For further explanation, see page 169 ff.

No. 29 exhibits an unusual condition in the presence, in the old part, of an unpaired complete mesentery. Other old mesenteries are somewhat unevenly developed.

NO.	OLD MESENTERIES						NEW MESENTERIES					REGENERATION		FIGURE
	B	N-D	D	N-D	D	B	B	N-D	D	N-D	B	Complete	Days	
23	c	cc	dd	cc,cc		c	*c*	*cc*	*dd*	*cc*	*c*	8		
24	c	cc	dd	cc		c	*none*							
25	c	cc	dd	cc		c	*c*	*cc*	*dd*	*cc*	*c*	8	56–62	
26	c	cc	dd			c	*c*	*cc*	*dd*		*c*	6		
27	(I)	cc,cc	dd	cc,cc,cc		c	*c*	*cc*	*dd*	*cc,cc*		7		
28	c	cc	dd	cc,cc,cc,cc		c	*c*	*cc*	*dd*	*cc*	*c*	8		
29	c	cc	dd	cc,cc,c	dd	c	*c*	*cc*	*dd*	*cc*	*c*	8		
30	(I)	cc	dd	cc		(I)	*six*					4	30	
31	(I)	cc	dd			c	*c*	*cc*	*dd*	*cc*	*(I)*	7		
32	(I)		dd			c	*c*	*cc*	*dd*	*cc*	*(I)*	7		
33	(I)	(1)?c	dd	cc		c	*c*	*cc*	*dd*	*cc,cc*	*(II)*	9		
34	c	cc	dd	cc,cc		c	*c*	*cc*	*dd*	*cc*	*c*	8		
35	c		dd			c	*six very small*						14	
36	c		dd	c		c	*six very small*							
37	c		dd			c	*c*	*cc*	*dd*	*cc*	*c*	0		
38	(I)		dd	cc,cc		*		*cc,cc*	*dd*	*cc*		2		
39	c	cc	dd	cc,cc		c	*c*	*cc*	*dd*	*cc*	*c*	8		
40	c	cc,cc	dd	cc,cc	dd	c	*c*	*cc*	*dd*	*cc*	*c*	8	30	
41	c	cc,cc	dd	cc,cc,cc		c	*two very small*							
42	c	cc	dd	cc,cc	dd	c	*c*	*cc*	*dd*	*cc*	*c*	8		
43	(I)			cc		c	*c*	*cc*	*dd*	*cc*	*(I)*	7		40
44	c					d	*d*	*cc,cc*	*dd*	*cc*	*c*	10		
45	(I)	cc,cc	dd	cc		c	*c*	*cc*	*dd*	*cc*	*(I)*	7		
46	(I)	cc,cc	dd	cc,cc		(I)	*two*							3,4,5
47	(I)		dd	cc		c	*c*	*cc*	*dd*	*cc*	*(I)*	6		
48	(I)	cc	dd	cc		c	*c*	*cc*	*dd*	*cc*	*(I)*	7		
49	(II)	cc	dd	cc,cc		(1)	*four*							
50	c		dd	cc		c	*c*	*cc*	*dd*	*cc*	*c*	8		
51	c		dd			c	*eight*					4		
52	c	cc	dd	cc,cc	dd	c	*c*	*cc*	*dd*	*cc*	*c*	8		
53	(I)	cc,cc	dd			c	*six small*							
54	(II)		dd			*		*cc,cc*	*dd*	*cc,cc*	*(II)*	10		

TABLE 7

This table shows the mesenteric forms of thirty-three specimens. Some of these were selected, in collecting, on the basis of the position of the new area or on other grounds that prevent their consideration in connection with some matters for which specimens given in tables 3 to 6 are available. Others were excluded from table 6 on grounds which have been stated in connection with that table and in the text (p. 169 ff). Still others are animals regenerating after artificial cuts. In the case of these last, the length of the regeneration period is given in the last column of the table. Since the period of regeneration is unknown for all other specimens listed in this table, the cut specimens may be identified by the presence of figures in the last column following their formulas.

Dashes in the columns for Old Mesenteries indicate that some part of the old material present at the time of the recorded division was removed by a later division or cut and the full formula of the old part is therefore unknown. In the old regions of these specimens only bounding mesenteries are recorded.

The case of No. 80 is discussed on page 211. Attention should be called to the fact that a new mesentery apparently of the first incomplete cycle mates with an old one of the second incomplete cycle. It is clear that no orange stripe was formed in the included endocoel.

NO.	OLD MESENTERIES						NEW MESENTERIES					REGENERATION		FIGURE
	B	N-D	D	N-D	D	B	B	N-D	D	N-D	B	Complete	Days	
55	c	cc	dd	cc		c	*c*	*cc*	*dd*	*cc*	*c*	8		
56	c		dd			c	*c*	*cc*	*dd*	*cc*	*c*	7		
57	c		dd			c	*c*	*cc*	*dd*	*cc*	*c*	8		
58	c	cc	dd	cc		c	*c*	*cc*	*dd*	*cc*	*c*	8		
59	c	cc,cc	dd	cc,cc		c	*c*	*cc*	*dd*	*cc*	*c*	8		
60	c	—	—	—	—	c	*c*	*cc*	*dd*	*cc*	*c*	7		
61	c	—	—	—	—	c	*c*	*cc*	*dd*	*cc*	*c*	4		
62	c	—	—	—	—	c	*c*	*cc*	*dd*	*cc*	*c*	6		
63	c	—	—	—	—	c	*c*	*cc*	*dd*	*cc*	*c*	8		14
64	c	—	—	—	—	c	*c*	*cc*	*dd*	*cc*	*c*	8		15,16
65	c		dd			c	*c*	*cc*	*dd*	*cc*	*c*	8		
66	(I)	cc	dd	cc		c	*c*	*cc*	*dd*	*cc*	(*I*)	7		12,13
67	(I)	cc	dd			c	*c*	*cc*	*dd*	*cc*	(*I*)	7		
68	(I)		dd	cc,cc	dd	c	*c*	*cc*	*dd*	*cc*	(*I*)	5	56	
69	(I)	—	—	—	—	c	*c*	*cc*	*dd*	*cc,cc*	(*I*)	7		
70	(I)	—	—	—	—	c	*c*	*cc*	*dd*	*cc*	(*I*)	7		
71	(I)	cc,cc	dd	cc		c	*c*	*cc*	*dd*	*cc*	(*I*)	7		
72	c	cc	dd	cc,cc	dd	(1)	(*1*)	*cc,cc*	*dd*	*cc*	*c*	9		
73	c		dd	cc		(I)	(*I*)	*cc*	*dd*	*cc*	*c*	7		19,38
74	(I)	cc				c	*c*	*cc*	*dd*	*cc*	(*I*)	7		

TABLE 7—*Continued*

NO.	OLD MESENTERIES						NEW MESENTERIES					REGENERATION		FIGURE
	B	N-D	D	N-D	D	B	B	N-D	D	N-D	B	Complete	Days	
75	(I)	cc,cc	dd	cc,cc		(I)	*(I)*	*cc,cc*	*dd*	*cc,cc*	*(I)*	10		
76	(I)	—	—	—	—	(I)	*(I)*	*cc*	*dd*	*cc*	*(I)*	6		
77	(I)	—	—	—	—	(I)	*(I)*	*cc,cc*	*dd*	*cc,cc*	*(I)*	10		
78	(I)	—	—	—	—	(I)	*(I)*	*cc,cc*	*dd*	*cc,cc*	*(I)*	10		
79	(II)	cc				(I)	*(I)*	*cc*	*dd*	*cc,cc*	*(1)*	8	49	
80	(II)		dd			(II)	*(I)*	*cc,cc*	*dd*	*cc,cc*	*(1)*	10		37
81	(I)	cc,cc	dd	cc,cc		(I)	*(I)*	*cc,cc*	*dd*	*cc,cc*	*(I)*	10		15,16
82	(II)					(II)	*(II)*	*cc,cc*	*dd*	*cc,cc*	*(II)*	10		37
83	*	cc,cc				*		*cc,cc*	*dd*	*cc,cc*		10	56	
84	*					d	*d*	*cc,cc*	*dd*	*cc,cc*		11	49	

TABLE 8

This table records the mesenteric formulas of five specimens with apparently unusual regenerated parts. These were excluded from table 7 only for convenience. For description see page 190. A possible partial explanation of these irregularities is given on page 190.

NO.	OLD MESENTERIES					NEW MESENTERIES							REGENERATION	
	B	D	N-D	D	B	B	N-D	D	N-D	D	N-D	B	Complete	Days
85	*	dd	cc,cc		d	*d*	*cc,cc*						5?	49
86	*	dd	cc,cc	dd	c	*c*	*cc*	*?*	*cc,cc*				7	54
87	*	dd	cc,cc	dd	*		*cc,cc*	*dd?*	*cc,cc*				10	54
88	*		cc		(I)	*(I)*	*cc*	*dd*	*cc,cc*	*dd*			10	56
89	*		cc		c	*c*	*cc*	*dd*	*cc,cc*	*dd*	*cc,cc*		15	60

TABLE 9

This table summarizes the facts presented in tables 3 to 6, on the left with respect to character of the spaces cut by the division plane, and on the right concerning the total number of complete mesenteries formed in the corresponding regenerating regions. On the right, specimens of table 7 are included, but those of the preceding tables which had not regenerated sufficiently to show their final mesenteric formulas are necessarily omitted. Only those cases are included which show clearly the final formula of the regenerating region, but they are included whether or not the potentially complete mesenteries were actually complete at the time of killing (p. 186).

On the left are indicated the classes into which the regenerating regions are grouped on the basis of the old bounding mesenteries. The 'frequencies' here given are the numbers of cases in each class found in tables 3 to 6, and may be taken to represent fairly well the proportions in which divisions of the different classes naturally occur. (See p. 177 for discussion.)

Figures in italics in the right half of the table represent for each class, as given on the left, the frequency of the numbers of complete mesenteries (indicated at the heads of the columns) found in tables 3 to 7.

OLD BOUNDING MESENTERIES		FREQUENCY	NUMBER OF COMPLETE MESENTERIES (5–11) WHICH HAVE BEEN REGENERATED						
			5	6	7	8	9	10	11
Two complete.....	Two non-directives.....	28		*2*		*25*			
	One directive, one non-directive..	4					*1*	*3*	
One complete.....Non-directives.....		32	*1*		*17*		*17*		
One incomplete.....	One directive, one incomplete non-directive.....	1							
Two incomplete non-directives.....		9		*1*		*3*		*7*	
One complete non-directive, one doubtfully incomplete..		3					*1*		
No unpaired mesentery on one side...	One complete non-directive.....	1					*1*		
	One directive.....	0							*1*
	One incomplete non-directive.....	5				*1*		*4*	
Unpaired mesenteries lacking on both sides.....		*0*						*1*	
(*See note*).....	One complete non-directive.....	1				*1*			
	One non-directive doubtfully complete.....	1			*1*				
	One directive.....	1							
	One incomplete non-directive.....	1						*1*	

NOTE. *On one side a new division has passed through the boundary between old and regenerating regions (p. 189).*

TABLE 10

This table deals, in its right and left halves, with the same material as the corresponding parts of the preceding table; but in the present instance each boundary and associated half of a regenerating zone is considered separately. On the left side of the table are summarized the facts represented in tables 3 to 6 in so far as these concern the character of the spaces through which the planes of division pass. The symbols in the column headed 'B' are the same as those in the columns of bounding mesenteries in those tables and have the same significance. In the column headed 'Frequency' are given the number of instances of the bounding mesenteries indicated on the left recorded in tables 3 to 6, and the sum of these frequencies is the total number of symbols in the columns of bounding mesenteries in those tables. Since each old bounding mesentery is separately considered here, the sum of the numbers in the column headed 'Frequency' is twice the sum of the numbers found in the corresponding column of table 9.

Omitting doubtfully complete endocoels, doubtfully incomplete endocoels, and the four examples indicated in the last row, we find the ratios and percentages of locations of the fission planes to be as follows: complete endocoels, 103 (62 per cent): incomplete endocoels, 57 (34 per cent): exocoels, 6 (4 per cent). The ratio of complete to incomplete endocoels, is 103: 57, or 64 per cent to 36 per cent.

On the right are given the frequencies of the numbers of complete mesenteries (at the heads of the columns) regenerated between the new directive plane and the bounding mesentery indicated at the left of the rows. This part of the table includes specimens from table 7 in addition to those of tables 3 to 6. Of necessity, it excludes such specimens as did not clearly show the final number of regenerated mesenteries. Because of these inclusions and exclusions the number of individuals represented in the two halves of this table are not the same. The total number of regenerated mesenteries represented in this table is the same as in table 9.

The average number of mesenteries added in the course of a division followed by regeneration may be obtained as follows: take the average number of mesenteries indicated in each horizontal row of the right half of the table (omitting doubtful rows) and multiply by the frequencies given on the left; add these totals and divide by the sum of the frequencies (omitting doubtful ones). This gives 4.2 as the average addition for a lateral half of a regenerating zone, or 8.4 per regenerating area.

NOTE. *The four cases, in the last row, designated by a dagger refer to no. 18b and no. 22b, table 5, in which specimens two successive fission planes have apparently cut away first one and then a second old bounding mesentery. (For fuller explanation see p. 189.)*

POSITION OF DIVISION PLANE			B	FREQUENCY	COMPLETE MESENTERIES REGENERATED				
					2	3	4	5	6
Endocoels	Complete	Non-directive	c	97	3		93		
		Directive	d	6				1	4
		Doubtfully complete	c?	1		1			
	Incomplete	First grade	(I)	42		24		22	
		Second grade	(II)	8				10	
		Third grade	(III)	4				4	
		Undeterminable grade	(1)	3				3	
		Doubtfully incomplete	(1)?	3				1	
Exocoels			*	6				9	
(*See note*)			†	4			2	1	

TABLE 11

This table shows the number of mesenteries reaching the esophagus in individuals represented in tables 3 to 6 upon completion of the recorded regeneration. Specimens are distributed according to the number of siphonoglyphs and the number of complete mesenteries. The figures in the body of the table represent numbers of specimens. Average numbers of mesenteries are given for each siphonoglyphic class and for the whole. The triglyphic specimen with nineteen complete mesenteries had five directives, one lacking a mate.

	NUMBER OF COMPLETE MESENTERIES AFTER REGENERATION															AVERAGE MESENTERIES
	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Monoglyphic	2				1											11.3
Diglyphic	2		14		5	1	8	1	5		2		3			15.0
Triglyphic									1	1	2	1	3			20.5
Tetraglyphic															1	24
Totals	4		14		6	1	8	1	6	1	4	1	6		1	15.8

TABLE 12

This table is taken, with some modification, from Carlgren's table ('09, p. 40) summarizing the results of his study of regenerating anemones at stages when the order of development of the mesenteries could be determined.

Roman numerals at the head of different columns represent the types of arrangement indicated by corresponding numerals in figure 35. In the horizontal rows are indicated the number of cases of each type found in regenerating pieces of different kinds.

Carlgren used, in this study, artifically cut pieces of Sagartia viduata; natural fragments of Metridium dianthus, and pieces similar in character artificially separated from the parent polyp; and naturally produced basal fragments of Aiptasia diaphana. Some pieces of Sagartia and Metridium were cut in such a way as to leave only basal tissue to form the new polyp. These are designated as 'basal pieces.'

In all of the ten cases of Type VIII in Sagartia the new directive plane is perpendicular to the plane passing through the middle of the piece before regeneration. In at least some of these specimens (and I presume in all, since otherwise the relation of the new directive plane to the old middle plane could probably not be determined) some old tissue was present, on one side, between the mesenteries I have designated as c^2 and c^3.

The single example of Type II in Aiptasia shows a second esophagus.

Type VIII is the one found by Cary in three species of Aiptasia and in 'Cylista leucolena' (?).

Regenerating specimens of S. luciae, when old complete bounding mesenteries are absent, are of Type III, V, or II.

		I	II	III	IV	V	VI	VII	VIII	IX	X
Fragments	Sagartia	26	48	15	7	2+1?			10		
	Metridium, natural			1		1	2				
	Metridium, artificial		3	9		4					
	Aiptasia		1						21		
Basal pieces	Sagartia	5	1	1?				1		2	
	Metridium	2	2	1							1

TABLE 13

This table indicates the numbers of specimens in lots collected at different times and from different localities, all at Woods Hole, Massachusetts. The specimens are classified according to number of siphonoglyphs. For an account of the methods of collecting these specimens and recording the data here given, see page 218 ff.

LOT	DATE	LOCALITY	MONO-GLYPHIC	DIGLYPHIC	TRI-GLYPHIC	TETRA-GLYPHIC	TOTALS
1	1909, July 12	Ram Island	2	77	7	0	86
2	1909, July 18	U. S. B. F. Wharf	0	44	4	0	48
3	1909, September 22	U. S. B. F. Wharf	16	69	11	3	99
4	1910, November 28	Penzance	15	182	10	0	207
5	1911, March 31	Penzance	19	134	7	0	160
6	1911, May 30	Gut of Cancer, smooth stones	13	118	4	0	135
7	1911, May 30	Pine Tree Island, smooth stones	5	94	8	0	107
8	1911, June 29	Gut of Cancer, smooth stones	14	129	2	0	145
9	1911, June 29	Gut of Cancer, rough stones	28	254	8	1	291
Totals			112	1101	61	4	1278

TABLE 14

This table shows, for four lots of individuals, the number of specimens recorded as having the indicated numbers of orange stripes. Lots 1 to 3 are the same as those of the same designations in table 13. Lot X is a selected group of unusually large specimens collected at U. S. B. F. Wharf, Woods Hole, Massachusetts, September 11, 1909.

These data are subject to most of the limitations stated for table 13 (p. 219).

The totals of this table (excluding lot X) are plotted in figure 42. For a discussion of the data, see page 221.

LOT	NUMBER OF STRIPES																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1		5	6	6	2	6	6	15	9	10	6	5	8	4	3	0	1	1	1	1		
2		1	0	3	1	4	2	8	3	8	3	3	5	1	2	1	1	1	0	0	0	1
3	1	0	2	2	2	5	1	7	6	4	7	8	13	4	10	5	9	1	1	2	0	3
Total	1	6	8	11	5	15	9	30	18	22	16	16	26	9	15	6	11	3	2	3	0	4
X							1	0	0	2	5	1	13	7	10	2	4	1	2	1	1	

PLATE 1

EXPLANATION OF FIGURES

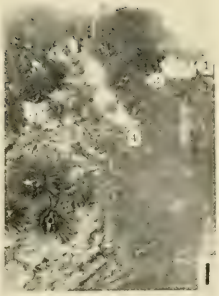
1 Colony of S. luciae photographed through the vertical sides of an aquarium. A number of specimens, such as that indicated at *1*, are ensconced in barnacle shells. The characteristic vertical orange stripes on the column do not appear in the photograph. White bars may be seen crossing the oral discs. These mark the positions of siphonoglyphs. Continuations of the radial white bars frequently border the oral aperture. The individual at *2*, has one radial white bar, a monoglyphic specimen. A triglyphic individual with three white bars may be seen at 3. The specimen at *4* has a minute radial projection of the white border which fringes the mouth, giving ground for suspecting some irregularity of internal structure.

2 A colony seen from above. *1*, as in figure 1. *2*, individuals each having but one prominent white bar, with extension partially surrounding the mouth, indicating a single well-developed siphonoglyph and, opposite this, a regenerating region in which a white line will eventually appear. *3*, a specimen with one broad white bar and one narrower and fainter. The latter is newly formed in a regenerating region.

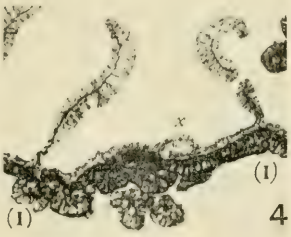
3 Microphotograph of cross-section of an anemone (no. 46, table 6) which had but one white bar and gave no external evidence of new tissue, being therefore apparently monoglyphic. The line of new tissue marked by the loop at *X* was probably mistaken for an orange stripe. For explanation of symbols, see page 232. × 32.

4 Photograph at greater magnification of the new part and adjacent structures of section shown in figure 3. × 60.

5 Similar photograph of the regenerating region of a more aboral section of the same specimen. The loop has opened and appears as two separate mesenteries, c^1, c^1. × 60.


1

2


x
(1)
(1)
4

d
x
(1)
(1)
3

(1)
(1)
(1)
5

PLATE 2

EXPLANATION OF FIGURES

6 Microphotograph of a section of a specimen (no. 1b, table 3) killed twenty days after division. The newest area lies between the two old bounding mesenteries, (II) and c, and contains six small mesenteries—c is complete orally. The greater part of the section is occupied by an older regenerating area, from (I) to (I) exclusive. Mesenteries labelled c^2 and c^3 and the mesenteries in corresponding positions on the opposite side of the directive plane of this area are complete orally. × 27.

7 Photograph at higher magnification of the newest area and its bounding mesenteries from the same section as shown in the preceding figure. × 56.

8 A regenerating area (from no. 3a, table 3) slightly more advanced than the newest part shown in figure 7, though actually younger (ten days). Bounding mesenteries, (I), both incomplete. In all, twelve new mesenteries can be counted in the sections of this specimen. × 60.

9 Far oral section of a specimen described when living as having three orange stripes and one white bar in the old region, and one white bar in a regenerating area which occupied approximately one-fifth of the circumference of the specimen. See diagram, figure 36. Typical regeneration following division in two complete endocoels. The characteristic size relation existing among the regenerating mesenteries is well represented. On each side a new mesentery, c^3, mates with the old bounding mesentery, c. The appearance of a siphonoglyph in section is shown at S in the new region and in the groove on the opposite side of the esophagus. In the latter the cilia, though distinguishable in the photograph, are not reproduced in the figure. In the upper part of the figure a fold of the oral disc appears. × 40.

10 Part of a more aboral section of the same specimen as shown in figure 9. The order of size of the new mesenteries is maintained. × 40.

11 Part of a section of an anemone (no. 8b, table 3) killed fifty-six days after division. On one side of the new directive plane only two potentially complete non-directives (c^1, c^2) formed where more commonly four are regenerated. Attention should be called to the probability that the mesentery c^2 in such a region of reduced number of new mesenteries corresponds with the one designated c^4 of a region regenerating the typical number of mesenteries (p. 191, 197). × 34.

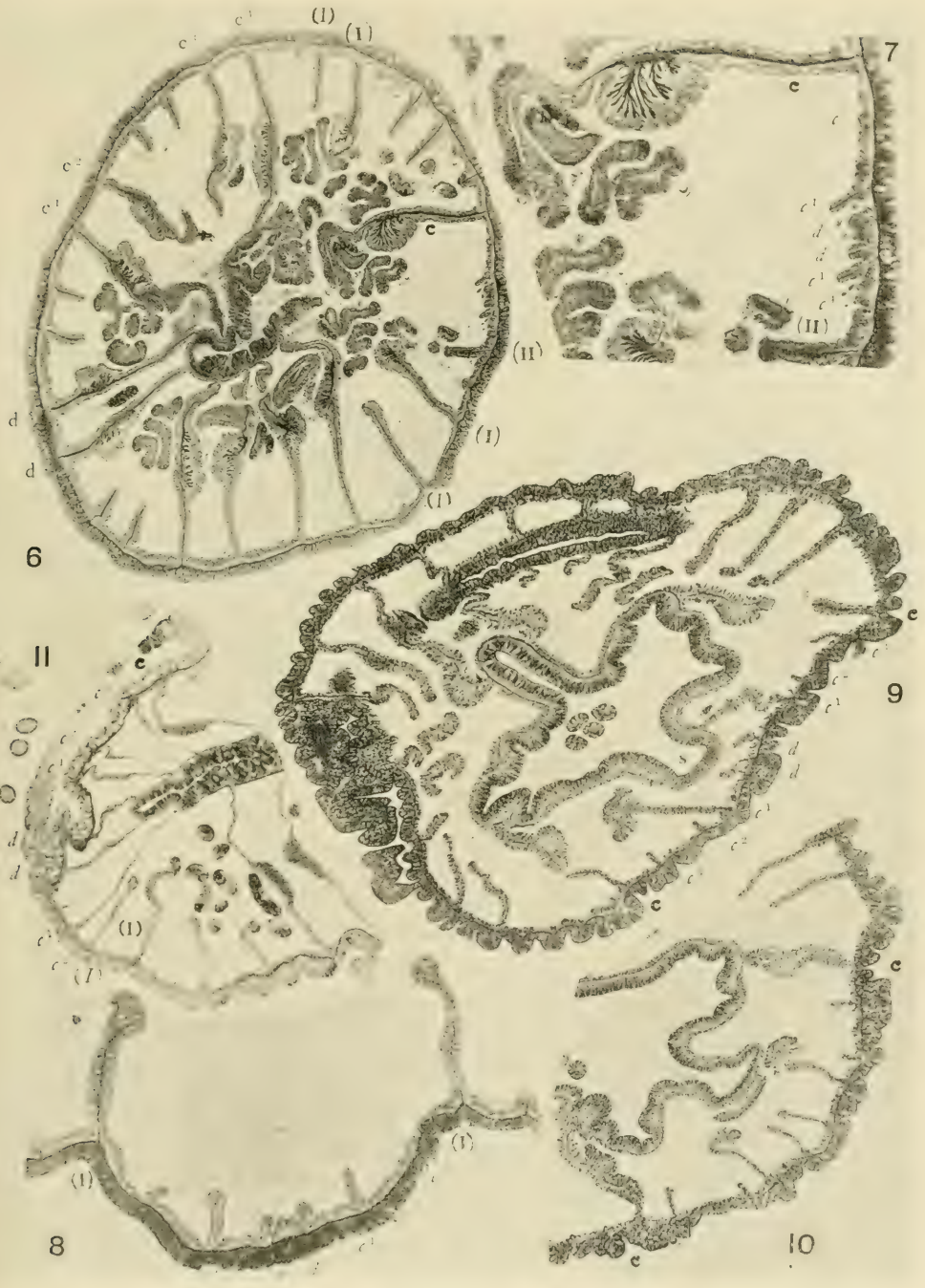

6
7
8
9
10
11
(I)
(II)
c
d

PLATE 3

EXPLANATION OF FIGURES

12 Section of a specimen (no. 66, table 6) which showed one prominent and one faint white bar, the latter lying in a sector of new tissue constituting one-fourth of the bulk of the animal. A fold of the oral disc (or of the column) appears on the side of the section occupied by the new mesenteries. As in the individual represented in figure 11, less than the typical number of regenerating mesenteries is present on the side of the incomplete bounding mesentery, (**I**). × 45.

13 Part of a more aboral section of no. 66. Size relations indicate the order of development of the new mesenteries. c^2 and c^3 are usually more nearly equal in size. The new incomplete bounding mesentery (*I*) appears here though lacking farther orally (fig. 12). × 45.

14 Section of a triglyphic specimen (no. 63, table 6). Externally there were seen, not quite opposite each other on the oral disc, two white bars lying within the limits of a darker zone forming the greater part of the specimen. On one side was a small lighter colored (newer) sector. There were apparently ten orange stripes of two ages in the older part and beginnings of orange stripes in the newer zone. The most recently formed part, between **c** and **c**, shows a typical regeneration following division in two complete endocoels. Two mesenteries on the left of the section between the old bounding mesentery, **c**, and the pair of directives show a peculiar condition strongly suggesting the position of an old division plane. An anomalous set of four mesenteries is seen at *X* (p 205). × 25.

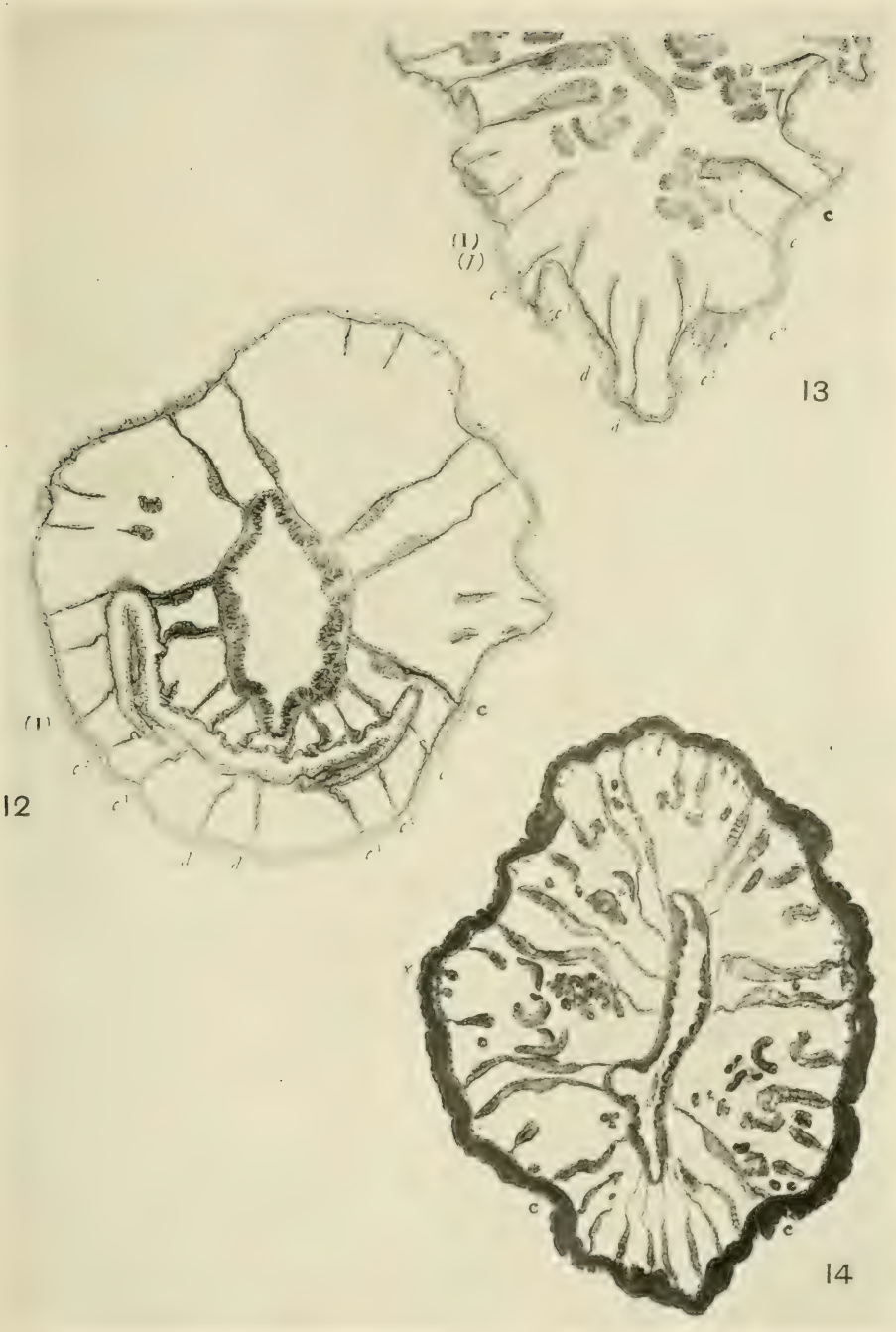

13
12
14

PLATE 4

EXPLANATION OF FIGURES

15 and 16 Sections of a very small and faintly colored specimen (later division recorded as no. 82, table 7; an earlier division as no. 64, table 7) having two white bars, but with no orange stripe and showing no external evidence of a division. Figure 15 a more oral section; figure 16 one taken below the aboral end of the esophagus. The sections indicate that the specimen is composed almost entirely of two regenerated areas. The older division plane passed through two complete endocoels as indicated by the size of mesenteries **c** and **c**. The later division occurred in incomplete endocoels, as is shown by the incomplete as well as the complete mesenteries. The new incomplete bounding mesenteries (*I*) and (*I*) of the later regenerating area may be seen in figure 16. Both figures × 60.

17 Section of a small individual (no. 82, table 7) with one white bar (monoglyphic) and ten evenly spaced orange stripes. There was no external evidence that a division had occurred. The sections make clear that somewhat less than one-fifth of the specimen, including no complete mesenteries, is old material, the remainder having regenerated following a division in two incomplete endocoels. Old bounding mesenteries (II) are incomplete and certainly not of the first incomplete grade. ×25.

18 Section of a small specimen which has certainly regenerated, as shown by mesenteries c^2 and c^3, but which gives no evidence as to the precise position of the plane of division. × 65.

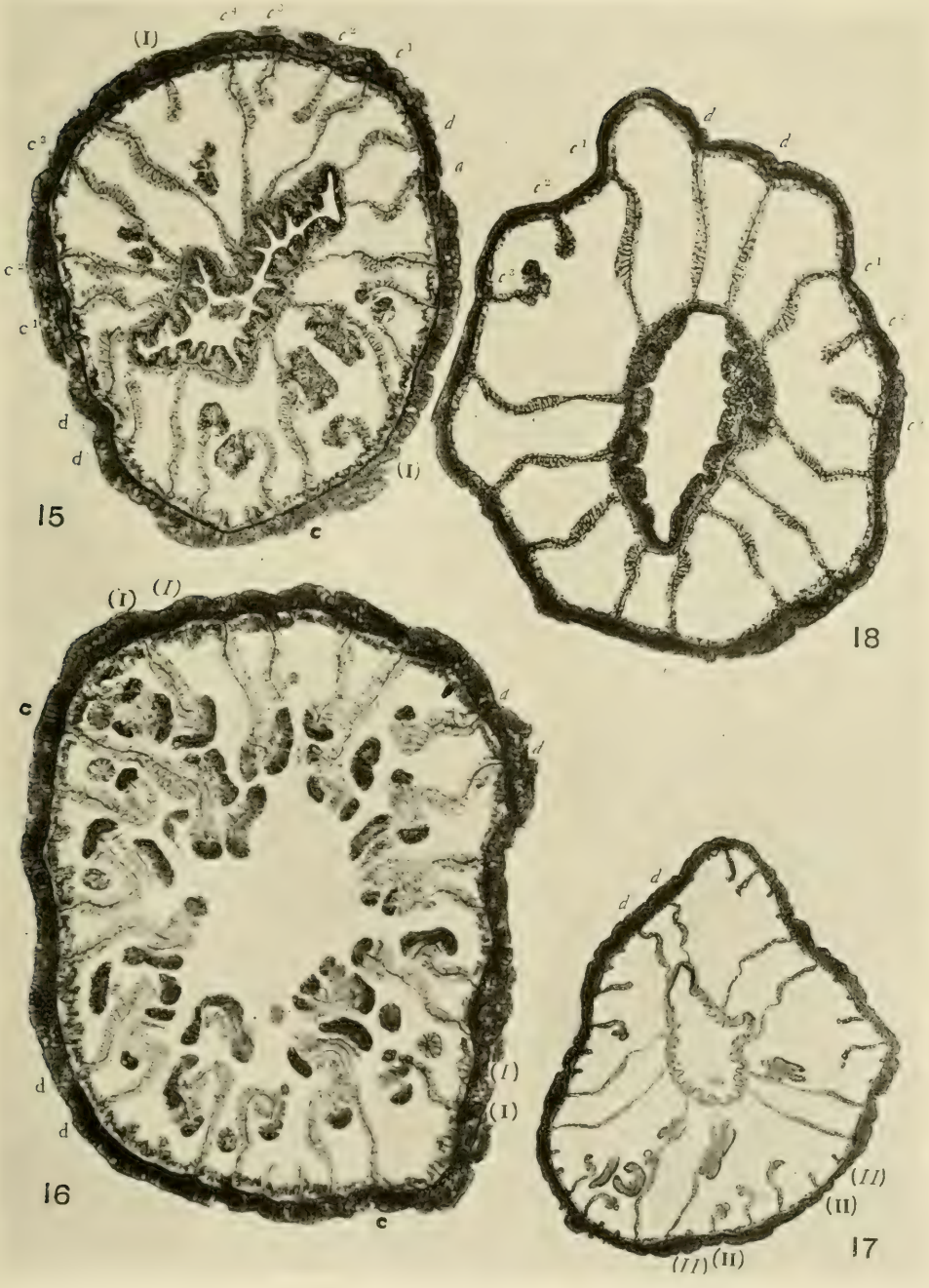

15
16
17
18
(I)
(II)
d
c

PLATE 5

EXPLANATION OF FIGURES

19 Section of an anemone (no. 73, table 7) composed of an older region containing a white bar and five more widely separated orange stripes, and a newer region with a slightly fainter white bar and seven less widely separated orange stripes. (Cf. diagram, fig. 37.) A cinclis is visible at *X*. × 20.

20 Section of a small specimen probably having undergone two regenerations as indicated by the character of the incomplete mesenteries. Precise positions of the division planes not certain. In the latest formed area (below), c^2 and c^3 on either side of the directive mesenteries are incomplete aborally. Possibly on either side the mate to c^3 is an old bounding mesentery; if new, the latest division occurred in exocoels. × 40.

21 and 22 Two sections of an anemone which showed, externally, slight irregularities in the distribution of its twelve orange stripes. These may have been due to unequal distention of different parts of the column wall. No certain external evidence of a division was present. Internally, as shown in these two figures, the greater development, in the upper region, of the incomplete mesenteries, especially those of the second grade, indicates that regeneration has occurred, but does not give any assurance as to the exact position of the boundary between new and old. Both figures × 25.

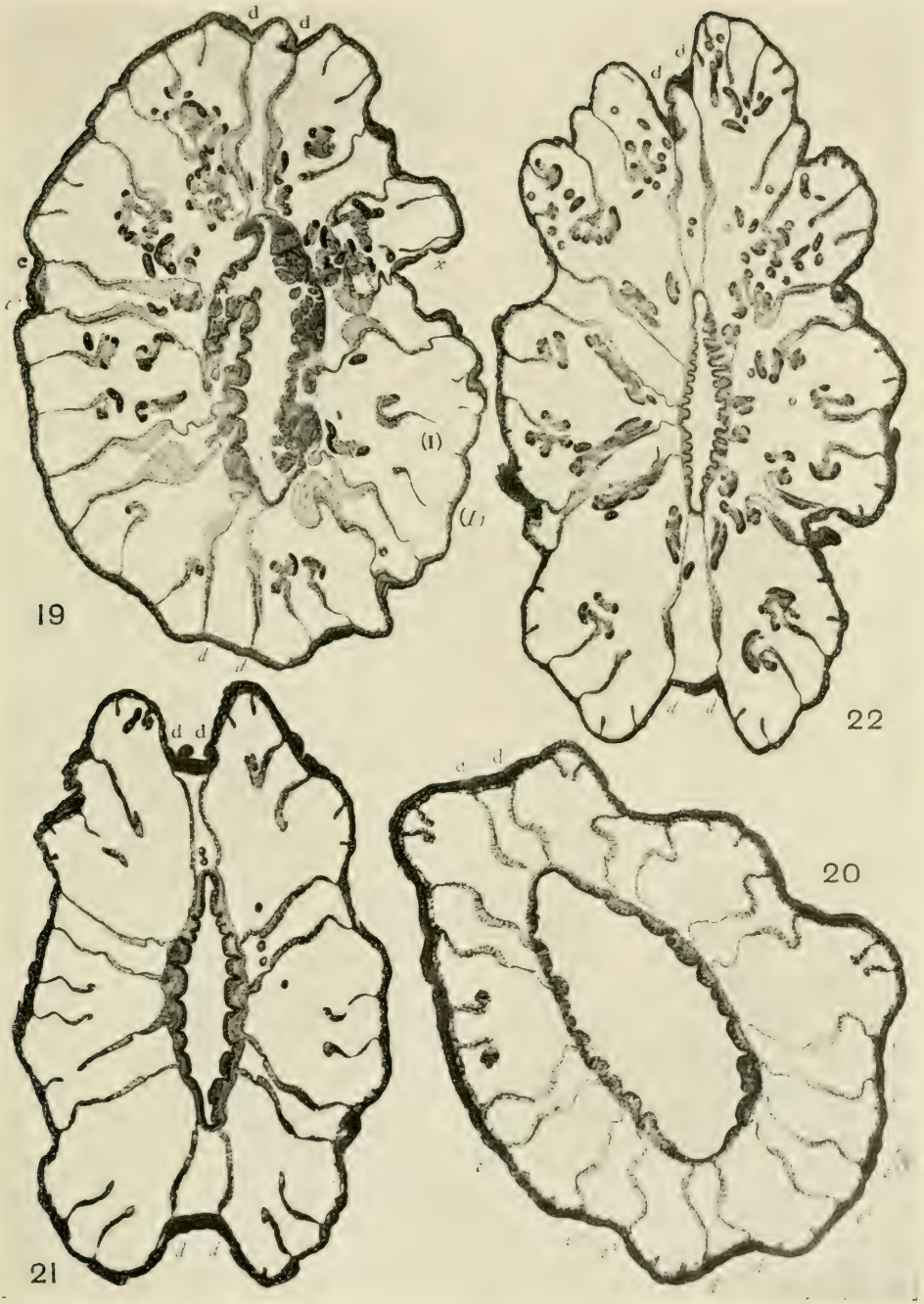

19
22
21
20

PLATE 6

EXPLANATION OF FIGURES

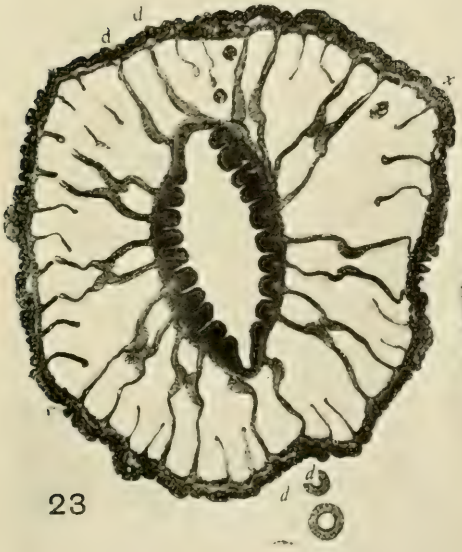
23 and 24 Sections of a specimen which showed no certain external evidences of division. The slight irregularity in the incomplete mesenteries shown at *X* in figure 23, and especially the irregularities in the incomplete mesenteries of the corresponding region in figure 24, indicate that two regenerations have occurred. The older is represented by the upper portion of the figures. The greater development of c, figure 24, shows it to be an old bounding mesentery of the older of the two divisions. × 35.

25 Section of a diglyphic specimen with two white bars and twelve orange stripes distributed as shown in figure 39. Sections showed no certain evidence of division, although the slightly greater development of incomplete mesenteries near the directives below suggests that this may be an older part. Pairs of complete non-directive mesenteries are here indicated by c. An acontium may be seen protruded through a cinclis in an incomplete endocoel of the second grade at a. A pair of mesenteries of the fourth incomplete cycle is present near the directives below. × 8.

26 Section of a perfectly regular diglyphic specimen with two white bars opposite each other on the oral disc and twelve orange stripes symmetrically placed and evenly spaced on the column. Internally no evidence of division. Pairs of directives are indicated by d. × 14.

25

26



23

24

PLATE 7

EXPLANATION OF FIGURES

27 to 32 represent two sections from each of three of the four parts into which specimen no. 19, of table 5, divided. No. 19, before this division, was a very large diglyphic specimen with twelve orange stripes and showing externally no evidence of a previous division. Old regions are indicated by a greater number of cycles of incomplete mesenteries, by the greater size of all mesenteries, and by the presence of gonads attached to the larger mesenteries. The position in the original polyp of the three specimens figured is indicated in table 5. The regeneration period was from 34 to 37 days. For a full account of this case see page 173. × 23.

27 A far oral section of no. 19a. The fission plane passed through one complete endocoel and one endocoel of the first incomplete grade. An unexplained irregularity is seen in the incomplete mesenteries of the old part near the complete bounding mesentery, c.

28 A more aboral section of no. 19a.

29 and 30 Respectively more oral and more aboral sections of no. 19d. Division occurred in incomplete endocoels, one of first cycle and one of third cycle. The small old bounding mesentery is in the position of one of the third incomplete grade and far aborally is similar in size to others of that cycle. Its new mate, barely indicated in figure 30, is smaller than the other new incomplete mesenteries, all of which are of the first incomplete grade. At present, therefore, it appears to belong to the second incomplete cycle and it is so indicated by the label. Probably its relation to the third cycle would be evident later.

30
(III)
(II)
d
d
d
d
(I)
(I)
29
(III)
(I)
d
d
(I)
(I)
28
27
c

PLATE 8

EXPLANATION OF FIGURES

31 and 32 Respectively more oral and more aboral sections of no. 19c. Two regenerating regions are represented. One of these follows a division in one complete endocoel and one endocoel of the first incomplete grade. This matches up with no. 19a (figs. 27 and 28). The other division occurred in one complete endocoel and one endocoel of the third or perhaps second grade (fig. 32). At some levels there is a mate to the incomplete bounding mesentery of this region, (III), which appears to belong to the second cycle of incomplete mesenteries.

33 Part of a section of an anemone fixed with protruded acontia, **a**. One lies in an endocoel of the second incomplete cycle and one in an exocoel. A third cinclis not occupied by an acontium is distinguishable at *x*. × 8.

34 A more highly magnified view of an acontium penetrating a cinclis lying in an exocoel or possibly in an endocoel with exceedingly slightly developed bounding mesenteries. × 43.

31
c
c
(I)
(I)
33
a
a
x
32
(III)
c
d
d
c
d
d
c
(I)
(I)
34

PLATE 9

EXPLANATION OF FIGURES

35 Diagrams I–VI, VIII, and X have been modified from those of Carlgren ('09, p. 39, fig. II). Diagram VII has been constructed from his description ('09, p. 35) of a specimen of Sagartia viduata (no. 15a7). Diagram IX has been taken from his figure ('09, Taf. 4, Fig. 47) representing a section of a specimen of the same species.

These diagrams show different arrangements of mesenteries found by Carlgren in regenerating specimens of Sagartia viduata, Metridium dianthus, or Aiptasia diaphana at stages giving indication of the order of development of the mesenteries.

Only mesenteries that would eventually be complete are represented.

Portions enclosed in dotted lines represent old material. Some mesenteries were present in every such region and, usually, one or more of the old mesenteries were complete. The two mesenteries shown here are not to be understood as indicating the number or character of old mesenteries.

I have labeled the new mesenteries in one-half of each diagram I, II and III with the symbols given in my figures and text to mesenteries occupying corresponding positions with respect to the new directive plane.

Carlgren's diagram 5 ('09, p. 39, Fig. II) shows mesenteries c^2 and c^3 on the left side bearing muscles facing each other instead of facing the mesenteries with which they commonly form non-directive pairs. The same arrangement is represented on both sides in his figure 37 ('09, Taf. 3). Since I find no mention in the text of so remarkable a condition, I am led to think that an error was made in both places. I have therefore changed the positions of these muscles in the corresponding diagram (III) of my figure. Certainly this coincides with the conditions in S. luciae.

For the frequency of these types of arrangement of mesenteries in regenerating pieces of the different species, see table 12.

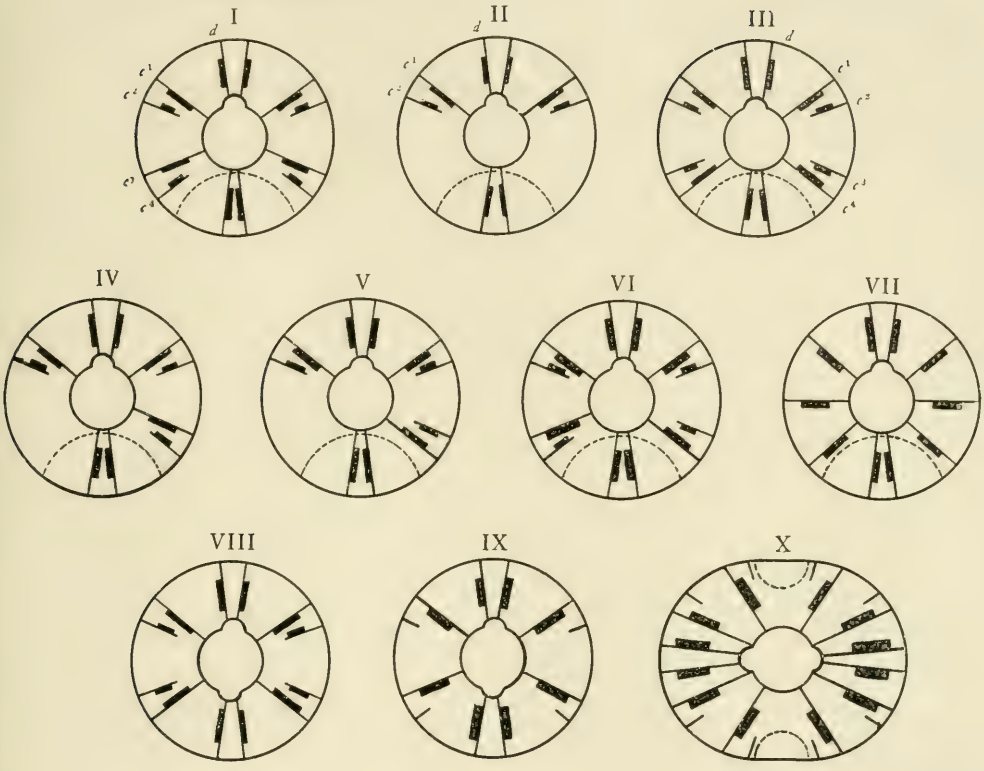
For a discussion of the relations of these types, see text, page 201.

36 to 41 Diagrams representing arrangements of orange stripes and white bars, as seen on living specimens. The inner part of each diagram represents the oral disc of an anemone with mouth in the center and with one or more white bars, shown by stippled lines, extending radially across the disc. The tentacular ring is not represented. In the outer part orange stripes are indicated by solid lines. Broken lines mark the boundaries between recently regenerated and older tissue. The distinction between these in living specimens showing early stages of regeneration is indicated by differences in color; in length, width, and density of the white bars; and in breadth, depth of color, and closeness of the orange stripes. The newer area is toward the lower edge of the plate except in figure 39, where it is above.

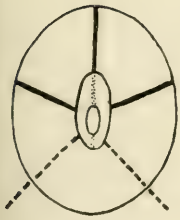
36 Diagram of specimen at a stage of regeneration when all of the complete mesenteries have formed but not all have reached the esophagus. A new white bar but no orange stripes have appeared. A section of this anemone is shown in figure 9.

37 A specimen (no. 80, table 7) in an advanced stage of regeneration. Two old and nine new orange stripes (the original record noted a possibility that one of the mesenteries close to the boundary might be old). For a description of internal structures, see page 211.

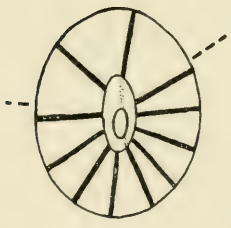
(*Continued on page 262*)



35



36



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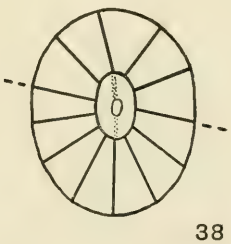

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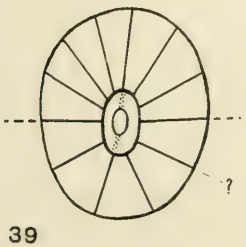

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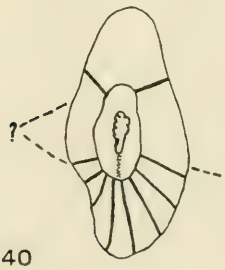

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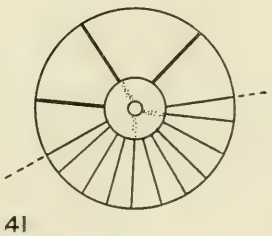


41

(*Continued from page 269*)

38 Specimen (no. 73, table 7) a section of which is seen in figure 19. The correspondence between external features and internal structures is typical, the distribution of orange stripes accurately indicating the positions of pairs of mesenteries of the first two cycles.

39. Specimen a section of which is shown in figure 25. Three days before this diagrammatic sketch was made, the orange stripes were noted as being equidistant. Internal structures give little suggestion of regeneration. The unequal distribution of the orange stripes shown in the diagram may be due to the momentary state of expansion.

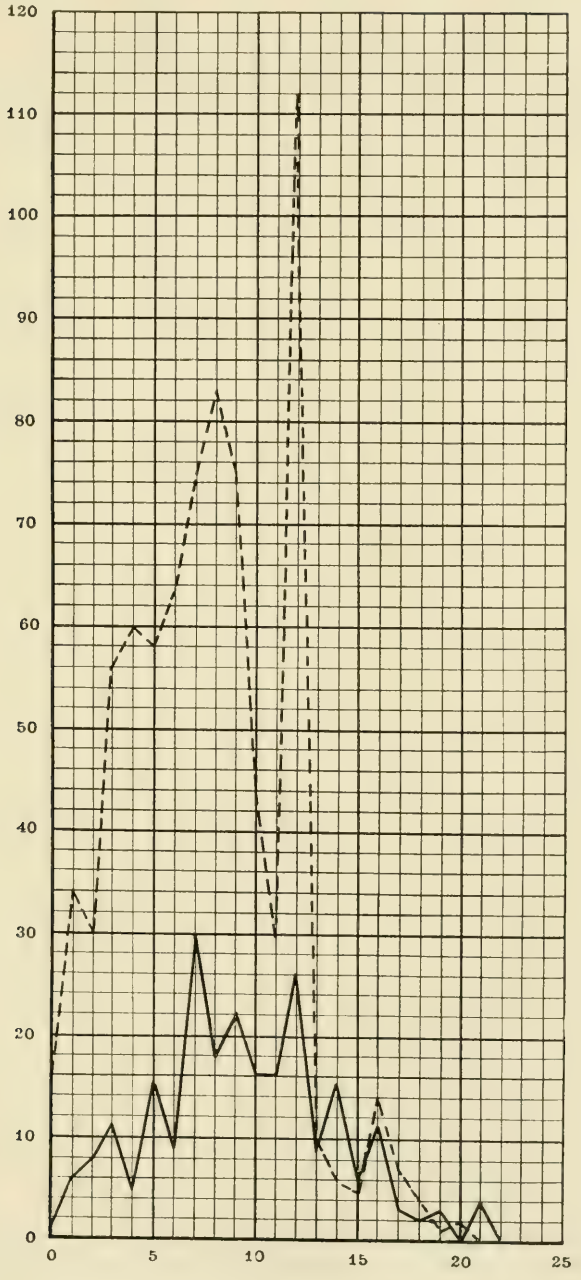
40 Diagram of a monoglyphic specimen (no. 43, table 6). Color difference did not clearly locate the boundary between old and new parts on the left. In sections the mesenteries gave clear evidence of its position.

41. Diagram of a triglyphic specimen with three old and eleven new orange stripes. Apparently the eleven orange stripes and two of the three white bars belonged to a single regenerating sector. Sections make it clear that there are here two regenerating zones of different age. See page 216.

PLATE 10

EXPLANATION OF FIGURES

42 Curve (solid line) representing the number of stripes in individuals of combined lots 1, 2, and 3 of table 14. The similar curve (broken line) given by Davenport ('03) is added for comparison. For a discussion of the data, see page 221.


120
110
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25

Resumido por el autor, Calvin B. Bridges.

La genética del color púrpura de los ojos de Drosophila melanogaster.

El color púrpura es una de las primeras mutaciones halladas en esta mosca (descubierta en 20 de febrero de 1912), la cual ha resultado especialmente útil. Es un caracter estrictamente recesivo, fácil y rápidamente separable del tipo salvaje, completamente viable, fértil y productivo. Su *locus* está situado en el segundo cromosoma, en un punto distante 6.2 unidades a la derecha, del locus del color negro y 52.7 unidades a la derecha del de la mutación estrella. La región indicada corresponde a la mitad del cromosoma, coincidiendo con lo que se pensó al planearle, puesto que esta región se caracteriza por un doble entrecruzamiento[1] anormalmente elevado, una sensibilidad especial a la acción de la edad, calor y frio sobre la cantidad de entrecruzamientos y por una limitación especial sobre la acción de ciertas variaciones genéticas originadas por el entrecruzamiento de los cromosomas. El color púrpura se ha utilizado para el desarrollo de muchos aspectos importantes de la genética de Drosophila; con el color bermellón produjo "intensificación" o "modificación desproporcionada." Ha servido también como modelo para la mutación "mímica" repetida, y ha sido también "recurrente." Finalmente, este color ha sido utilizado muy intimamente en el análisis del ligamiento autosomal (acoplamiento F_2, cruzamiento retrógado del macho y hembra para comprobar el entrecruzamiento, planeado de dos y tres puntos etc.). La curva de coincidencia correspondiente a la variación de la edad es, en términos generales, la imágen de la curva de entrecruzamiento para la variación de edad, mientras que la curva de coincidencia de la variación de temperatura parece limitarse a una linea recta independiente de la temperatura. Estas dos variaciones dependen, aparentemente, de dos factores fisiológicos independientes que afectan a la "longitud internodal" y al "coeficiente de entrecruzamiento," respectivamente.

Translation by José F. Nonidez
Columbia University

[1] Con esta palabra traducimos la correspondiente inglesa "crossing over" entendiéndose que se refiere a los cromosomas exclusivamente. "Acoplamiento" puede servir para traducir la palabra "coupling" y "ligamiento" para "linkage," a falta de otras mejores què sugieran la idea expresada por las palabras inglesas, que tal vez convendría conservar sin traducir. N. del T.

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THE GENETICS OF PURPLE EYE COLOR IN DROSOPHILA

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INTRODUCTION

Of the two hundred or more mutations of Drosophila, 'purple' ranks high among those that have proved especially useful because of their ease of identification or other excellent characteristics, and

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because of their favorable location in the chromosome. Purple has an even higher interest because of its connection with the development of several new fields in genetics and of principles that are now made use of in every Drosophila experiment.

ORIGIN

In a stock which was supposed to be simply vestigial there was found, February 20, 1912, a single male which had an eye color much like that of the well-known double recessive vermilion pink. The color of the vermilion-pink eye is about that of the pulp of an orange, and the early papers accordingly referred to this double recessive as 'orange.' The new color was seen to differ slightly from vermilion-pink in that it was of a brilliant ruby-like transparency, and lacked the flocculent or slightly cloudy appearance of vermilion pink. This difference seems to arise partly from a difference in the distribution of the pigment. In vermilion pink the pigment looks as though it were mainly in the spaces between the radially arranged ommatidia with a clearer zone just under the surface of the eye. One sees in the vermilion pink eye a light fleck which travels over the eye as it is turned. This seems to be due to a deficiency of pigment in the deeper parts of the eye, and the light fleck is this light center seen through the small group of facets whose axes are in line with the eye. The pigment in the case of the new eye color gave the appearance one would expect if it were uniformly distributed or even in solution throughout the eye.

INHERITANCE

This single male with the orange-like eye color was outcrossed to a wild female, and in F_1 gave only wild-type males and females (wild-type ♀ 32, ♂ 33; reference no. B1) which showed that the color was recessive. In F_2 the orange-like color reappeared, but in addition the sex-linked eye color vermilion emerged, and also a new eye color, 'purple,' which appeared equally among the F_2 females and males, and therefore was known to be an autosomal (not sex-linked) character. It was now evident that the

orange-like color resembled the old 'orange' (vermilion pink) genetically also, for it was proved by this F_2 to be a double recessive, vermilion purple, in which purple corresponds to pink.

It seems probable that the two eye-color mutations, vermilion and purple, present in the male first found were not of simultaneous or related origin. There was a vague report that the vestigial stock had contained vermilion at some time previous to this discovery. No vermilion or purple was found in it subsequently, however.

DESCRIPTION[1]

The purple eye color passes, in its development, through an interesting cycle of changes closely parallel to those seen in the ripening of a 'sweet' cherry. In the pupa the eye is at first colorless, then it assumes a creamy tone, which in turn becomes pinkish, passing progressively through a yellowish pink to pink and to ruby. When the flies hatch the color is a transparent rather deep ruby. This color rapidly deepens to garnet and then passes on to a purplish tone. The typical purple color at its maximum development—in flies about a day old, while retaining much of its transparency, appears darker in tone than the red of the wild type, purple being the first of such 'dark' eye colors. As the fly becomes older this 'ripe-cherry' color is progressively obscured, apparently by an increase in a flocculent red pigment like that of the wild fly. The eye color thus becomes somewhat lighter than red again, though always distinguishable by a lesser opacity and by a light 'fleck' in place of the hard dark fleck seen in the wild eye. With extreme old age the color approaches still closer to red, but does not become strikingly darker, as do pink and sepia, for example. In purples of the same age fluctuations in color are not great. The separation of purple from red is easy if done while the flies are mostly under two days old, though the climax in the development of the purplish tone offers the most favorable stage.

[1] For a colored figure of purple see plate 5, figure 8, of a forthcoming Carnegie publication (No 286) by Bridges and Morgan.

THE DIFFERENTIATION OF PURPLE BY VERMILION—DISPROPORTIONATE MODIFICATION

While the difference between the color produced by the purple gene and the color produced by its wild-type allelomorph (red) is distinct, it is neither great nor striking, since in tone purple is first slightly darker and later somewhat lighter than red. However, in classifying the eye colors in F_2 from the cross of vermilion by wild, it was observed that the difference between vermilion purple and vermilion not-purple was not only constant in direction, but also conspicuous in extent. The separability of purple versus not-purple is favored by the presence of vermilion, which may therefore be called a 'differentiator' of purple. Regarded in the converse relation, namely, the effect of purple on vermilion rather than the effect of vermilion on purple, purple is a much stronger modifier of vermilion than of not-vermilion. Purple may be described as a 'disproportionate modifier' of vermilion, since from the small amount of its effect on eye color when acting alone one would not have expected the great effect it produces when acting in the presence of vermilion.

This type of intensification—disproportionate modifier and, conversely, differentiator—stands midway between the normal relations where combination effects are roughly proportional to the separate effects so that both genes may be called 'general modifiers,' and the special relation where a given gene, 'specific modifier,' produces by itself no visible effect whatever, but which gives a more or less marked effect when acting in conjunction with some other gene, its specific base, sensitizer, or differentiator.

In order to make full use of this differentiation of purple versus not-purple by vermilion, it is necessary that all flies used in the experiment should be made homozygous for vermilion. This is often inconvenient, and accordingly only in the early and comparatively simple experiments was this method employed. It was soon found also that the separation of purple from red was not causing any trouble, so that the differentiation in this case has little net advantage, though it is still of interest as being the first example in Drosophila in which intensification was recognized and deliberately made use of.

THE RELATION OF PURPLE TO PINK

Some of the first purples which emerged in the F_2 were crossed to pink, to test whether these two somewhat similar eye color were allelomorphic or not. Four such pair matings produced only wild-type males (134) and females (137), which showed that purple is not an allelomorph of pink.

THE LINKAGE OF PURPLE AND VESTIGIAL

It was observed (April 2, 1912; B1) that in the F_2 from the cross of the original male to wild nearly all of the flies that were purple were also vestigial. This observation, following on the heels of the black-curved case, furnished a second example of autosomal linkage, this time one of so-called 'coupling,' the black-curved case having been 'repulsion.' No full counts were made of the proportion of purples that were vestigial. Indeed, at this early stage the linkage relations were receiving less attention than eye-color 'series.'

BACK-CROSS TEST OF MALES, PURPLE VESTIGIAL 'COUPLING'

The advantages of the back-cross method of testing linkage and the amount of crossing over had only begun to be appreciated. This method had been applied to a few cases in the X chromosome, and the general attack upon the linkage of all autosomal mutations planned by Sturtevant and Bridges (March 5, 1912) contemplated its full use. Thus far only two autosomal back crosses had been completed—those by which Sturtevant showed the absence of linkage between the second chromosome and the third chromosome (balloon ebony, May 10, 1912, and black pink, May 12, 1912). Because of the difficulty of getting the necessary double recessives no back cross which involved autosomal linkage had been possible until purple arose in the vestigial stock and thereby gave the required double recessive, purple vestigial, with which such a test of the amount of crossing over between purple and vestigal could be conducted. From the F_2 described above, matings were made which gave two stocks to be used in this test. One stock was the simple

purple vestigial, and the other was purple vestigial pure for vermilion. The special advantage of this latter stock lay in the fact that the presence of vermilion accentuates the difference in eye color between the flies that are purple and those that are not, that is, vermilion purple is easier to separate from vermilion than is the case in the equivalent separation of purple from red.

This latter stock was accordingly used in the P_1 mating for the first back-cross test. Vermilion purple vestigial males were outcrossed to females of vermilion stock (May 25, 1912). Both parents were homozygous for vermilion, and the F_1 flies were all vermilion as expected. Both purple and vestigial are recessive. When the back-cross matings came to be made, the culture bottle happened to contain no virgin F_1 females, since the P_1 mating had been made at Columbia and the F_1 progeny used had hatched en route to Wood's Hole. The back cross was therefore made in only one way—by mating the F_1 males to virgin vermilion purple vestigial females of the stock kept for that purpose. Five back crosses were started by mating in each case a single F_1 vermilion male by two or three stock vermilion purple vestigial females. At the end of ten days the parents were removed from the culture bottles and were put in fresh bottles in which second broods were raised. In one case a third brood was raised (table 1).

The linkage results of these back crosses were somewhat unexpected, for in four of the lines no crossovers at all were obtained, and in a fifth only a few. In the original F_2 culture several crossovers had been noted, and five F_2 cultures raised from the brothers and sisters of these back-crossed males were giving in the neighborhood of 15 per cent of crossovers (table 2). The apparent crossovers had all appeared in one culture of the first and of the second broods, and for this reason a third culture was raised from that particular set of parents and it also gave apparent crossovers.

A second back-cross experiment, using the simple purple vestigial stock instead of the vermilion purple vestigial, was started (June 25, 1912) a month later than the first and before

TABLE 1

The B. C. offspring given by the F_1 (vermilion) sons, from the outcross of (vermilion) purple vestigial males to vermilion females, when back crossed to (vermilion) purple vestigial females First and second broods given separately.

1912, JUNE 24[1]	NON-CROSSOVERS		CROSSOVERS	
	(Vermilion) purple vestigial	(Vermilion)	(Vermilion) purple	(Vermilion) vestigial
B10.1	90	186	0	0
	71	202	0	0
B10.2	72	197	0	0
	72	206	0	0
B11.1	45	126	0	0
	65	195	0	0
	51	88	7	3
B11.2	98	178	27	2
	43	72	4	0
B11.3	54	191	0	0
	37	70	0	0
Total	698	1711	38	5

[1] Date on which the cultures of the table began to produce offspring.

TABLE 2

The F_2 offspring given by the F_1 (vermilion) sons and daughters from the outcross of (vermilion) purple vestigial males to vermilion females

1912, JUNE 17	(VERMILION)	(VERMILION) PURPLE VESTIGIAL	(VERMILION) PURPLE	(VERMILION) VESTIGIAL
B8.1	200	23	9	5
B8.2	88	21	3	4
B8.3	255	66	25	5
B9.1	368	19	3	7
B9.2	346	17	19	9
Total	1257	146	59	30

the results of the first were fully known. A purple vestigial male outcrossed to a wild female produced wild-type sons and daughters (page B39; + ♀ 15, + ♂ 10). Four of the F_1 females were back crossed each by two or three purple vestigial males from stock. In this case F_1 females happened to be chosen because, as is usually the case, they hatched somewhat earlier than their brothers in the same culture.

These back-cross cultures (table 3), in common with the previous F_2 cultures (table 2), showed a fair amount of crossing over between purple and vestigial. A calculation showed that the percentage of crossing over was 9.1.

TABLE 3

The B. C. offspring given by the F_1 daughters, from the outcross of a purple vestigial male to a wild female, when back crossed to purple vestigial males

1912, JULY 16	NON-CROSSOVERS		CROSSOVERS	
	Purple vestigial	Wild type	Purple	Vestigial
B36.1	82	163	12	15
B36.2	80	133	14	10
B39.1	32	53	3	7
B39.2	62	141	9	9
Total	256	490	38	41

This was recognized as being of a different degree from the apparent percentage of 1.8 calculated from the first back cross (table 1). It was now realized for the first time that the two back crosses had differed in the sex of the F_1 flies tested by the back crosses—that the first back cross was a test of the amount of crossing over in the male and the second was of crossing over in females. Up to this time there had been no suspicion that the result of a back cross could be in any way dependent on the sex of the F_1 parent used in the experiment. From this evidence it was concluded that there was crossing over in the male, but that it was of different degree from that in the female. In September, 1912, Morgan showed that in the case of black vestigial no crossing over whatever had occurred in the male, while in the female there was even more crossing over than had been found

in the case of purple vestigial. Subsequent tests, including hundreds of thousands of individuals, have shown that ordinarily there is no crossing over in the male for any chromosome and that the few cases that have occurred were probably not brought about by the same mechanism as that by which crossing over is ordinarily effected.

NO CROSSING OVER IN THE MALE

A clear conception of the fact of no crossing over in the male was prevented in the original vermilion purple vestigial back-cross test by the apparent occurrence of crossovers in one of the five lines. No tests were made of the apparent crossovers because there was at that time no evidence, aside from the inconsistency within the experiment, to suggest that they were highly unusual. Against the supposition that some clerical error might have been made is the strong internal evidence presented by the aberrant cultures. Thus, the cultures could not have been F_2's that were mislabeled, since the proportion of purple vestigials in this line is the same as that in the other back-cross cultures and is much larger than that in any of the F_2 cultures. Also, the parents were carefully examined when they were transferred to the third culture bottle and were seen to be a vermilion male and vermilion purple vestigial females only, which is the back-cross type of mating. The examination of the parents also excluded the supposition that the line may have been a back-cross test of the female rather than of the male. Perhaps some unknown peculiarity of the stocks used may have been responsible for the apparent crossing over. Thus, it has been suggested that some other eye color resembling purple, such as 'maroon,' had been present, probably only in heterozygous form, in the vermilion purple vestigial stock. Such an explanation would account for the crossover class classified as vermilion purple, but entirely fails to account for the complementary class of exceptions—the few but carefully attested vestigials that were not-purple. In fact, none of the suggestions that have been made have offered a satisfactory escape from the alternative of some kind of crossing over in the male.

If these were true crossovers, it is possible that their production had no relation to the mechanism by which crossing over is ordinarily effected. Thus, Muller ('16) reported a case of crossing over in the back-cross test of a certain F_1 male from the mating of truncate to black. However, all of the gametes of this particular F_1 male proved to be crossovers, so that crossing over must have occurred, once for all, in an early cell of the embryo, and, as usual, no crossing over whatever occurred during spermatogenesis. The spermatozoa, all of which were descended from this embryonic crossover cell, simply inherited the crossover combination. In the case of purple vestigial, a like explanation would apply, except that in this case the crossing over occurred in a somewhat later stage of the embryo, and in consequence only a part of the spermatogonial cells carried the crossover combination and only sperm decended from these particular cells produced crossover progeny.

That somatic crossing over has little analogy to the ordinary type is proved by a similar case of embryonic crossing over in the female, which was then followed by crossing over of the ordinary type. A mating was made such that a certain class of daughters should all have the composition $\frac{v}{+}\frac{l_9}{+}\frac{+}{s}\frac{+}{g}\frac{B}{+}$.[2] Seven of the eight daughters tested had this expected composition, but one (no. 3464) gave only offspring corresponding to the composition $\frac{v}{+}\frac{+}{l_9}\frac{+}{s}\frac{+}{g}\frac{B}{+}$. That is, the gene for lethal 9 was found to be not in the chromosome in which it entered the zygote, but in the homologous chromosome derived from the other parent. As in the truncate × black case, this transmigration took place after fertilization and so early in the embryonic history that all the germ cells were descended from this altered cell. A significant feature of this case is that while the change must be described superficially as double crossing over,

[2] The symbols above the line represent the genes in the chromosome derived from one of the two parents, those below from the other. The + signs represent simply the wild-type allelomorphs of the mutants dealt with, and these signs may usually be omitted.

this double crossing over occurred within a region only ten units long—a space shorter than that in which double crossing over of the ordinary type has ever been detected even in certain regions of the autosomes in which double crossing over is relatively most frequent.

MUTATIONS

Two new mutations were found and two old ones reoccurred in these back-cross experiments on the linkage of purple and vestigial.

'Kidney' eye shape, a third chromosome recessive, was found in B. C. culture B10.2, June 26, 1912 (table 1). This mutant, the first affecting the shape or texture of the eye, was considerably used in the early days (Morgan, '14, and Bridges, '15), but has now been superseded by mutants less variable and easier to classify.

In culture B39.2 it was noticed, July 26, 1912, that several of the wild-type flies had more dorsocentral bristles on the thorax than the regular number, four. Later it was found that such extra-bristled flies were occurring in small proportions in all four sister cultures, from which it would appear that the mutation was a recessive, introduced through the purple vestigial stock used twice in the experiment. The extra bristles occurred among all classes in the experiment indifferently, which would seem to indicate that the gene were not second chromosome, since if it were the extras should have been relatively more frequent among the purple vestigials. The number of extra bristles varied from one to four, the highest total bristle number observed being eight. Extra bristles were also observed to be frequent in two or three other stocks. A stock throwing extra thoracic bristles derived from B39.2 was maintained by careless mass selection for some time and was finally given to Mr. E. C. MacDowell to be used as the basis of rigorous selection experiments (MacDowell, '15). As the result of a survey of all stocks known or suspected to contain extra bristles, MacDowell chose a certain wild stock as the most favorable starting-point for his selection.

In culture B9 a jaunty (jaunty 4) appeared which gave rise to a stock similar to the original jaunty, but so far as known of separate origin.

In three or four of the cultures, for example, in B9.1, arc wings (arc 6) appeared, and these were indistinguishable from the original arc, though quite certainly of different origin.

Since these early experiments many other mutations have arisen in experiments involving purple, but these need no special mention here.

THE INVIABILITY OF VESTIGIAL—PREMATURATION, REPUGNANCE, LETHALS

One of the most striking features of these crosses involving purple and vestigial was the failure of vestigial to appear in as high a proportion as expected. In the F_2 (table 2) where 25 per cent of the flies were expected to be vestigial, only 12 per cent were vestigial; in the back crosses where half of the flies were expected to be vestigial, only 29 per cent (table 1) and 36 per cent (table 3) were vestigial. That is, only about half as many vestigials as were expected appeared in these back crosses.

Such a condition is usually described by the blanket term 'inviability;' but a consideration of the 'inviability' met with in the case of rudimentary (Morgan, '12) had just led to two new conceptions: first, that the power of fertilization possessed by a given gamete is influenced by its genetic environment prior to maturation; second, that a given type of gamete is less likely to produce a viable zygote with one than with another of two classes of sperm. The conception of 'prematuration' was used to account for the fact that a rudimentary-bearing egg from a pure rudimentary female is much less able to give a viable offspring than a like egg from a mother only heterozygous for rudimentary. The principle of 'repugnance' was exemplified by the cross of rudimentary by rudimentary, which gave no offspring whatever though repeated several hundred times and although both the male and female give offspring when outcrossed.

The shortage of vestigials in the above crosses was thought to be parallel to the results given by rudimentary, except that in the

case of vestigial the effects of prematuration and repugnance were not as great in degree. On the basis of these results, an analysis of the extent to which each of these principles contribute to the 'inviability' of vestigial was undertaken by G. L. Carver (results not yet published). In Carver's investigation it was assumed that the shortage in these experiments had been largely due to a cause intrinsic to the vestigial itself, for which reason any stock of vestigial should be equally valid for the test. The stocks used in the above experiments were not used because they were full of odds and ends of mutations which might lead to confusion. The tests of Carver showed that very little prematuration or repugnance is inherent in vestigial, the ratios being exceptionally close to Mendelian expectation. Wherefore it seems probable that the shortage met with in the purple vestigial experiments was due to some cause peculiar to the stocks used or to the culture methods used in the experiment. Later tests with stocks descended from these original stocks have failed to give such aberrant viability.

Another explanation, that has been more recently applied to particular instances in which a character ordinarily of excellent viability has not appeared in the expected proportion, in that a lethal gene is present. Thus, an autosomal lethal in the second chromosome and quite far to the right of vestigial (i.e., close to speck) would give results roughly comparable to those observed. The difficulty with such an explanation in this case is that the uniform results given by all the cultures would require the lethal to be present in nearly all the individuals—a frequency entirely out of the question both from a priori considerations and from the results of subsequent tests made with these stocks. Such lethals as have been found in the vestigial stock (by L. J. Cole and by students at Columbia) should not give results like those observed.

THE PURPLE 'EPIDEMIC,' 'MUTATING PERIODS'

Shortly after the discovery of purple, purples or eye colors closely resembling purple began to be found in stock and experiments everywhere. In the interval of six months following the discovery of purple such occurrences numbered fourteen and furnished the first as well as the most striking of the 'epidemics of mutation' that seemed to sweep over our material at this period. From later and well-authenticated cases (e.g., vermilion, cut, notch, etc.) it appears that certain mutations do recur, and in the case of cut, four independent occurrences followed one another so closely that the term 'epidemic' is descriptive of the condition observed. However, in the early cases (purple, jaunty, arc, etc.) it is certain that a large majority of the apparent cases were not true reoccurrences of the mutative change, but were due to several other conditions. Thus, the 1st, 5th, 6th, and 13th apparent purples proved to be maroon, a third chromosome eye color practically indistinguishable from purple in appearance. That is, 'mimic' mutations were not at first distinguished from the original type. Nor were new mutant allelomorphs distinguished from types already known unless the difference was striking. Certain others of the occurrences were proved not to be of independent origin, thus purples 8 and 9 were both shown to have been descended from a certain common stock, and purples 10 and 11 were traced to a second common stock. It is undoubtedly true that in many cases where no connection can be traced such connection really existed, especially in the case of recessives, which might be distributed without giving sign of their presence. The psychological element, too, is important—it is exceedingly difficult to recognize a mutative change, even a striking one, before one becomes 'sensitized' to that particular mutation. Some of our mutant characters had long been present in stocks or experiments so that many flies showing the character must have been seen before attention became sharply focused upon the differences shown. Contamination and errors of one sort or another have also inflated the number of apparent reoccurrences of mutations. It is therefore to be doubted if more than two of the apparent reoccurrences of purple were genuine remutations.

REPETITION OF THE PURPLE VESTIGIAL BACK-CROSS TESTS

Because of the number of disturbing conditions that had been met with in the first set of tests of the linkage of purple and vestigial, a second and more extensive set was started. These second experiments were carefully planned, and in the results obtained approach present standards of uniformity and reliability. The viability of vestigial was excellent, and the equality of contrary classes throughout the experiments speaks for the favorable culture conditions. The new experiments were conducted with a purple vestigial stock descended from that used in the experiments of table 3, but cleared of mutations and perhaps other disturbing factors by outcrossing to wild and by selection started among the F_2 progeny and maintained for several generations until it seemed probable that the stock was clean. Also, from the progeny of table 3 some purple (not-vestigial) crossovers were selected, and from them was secured in a few generations a simple purple stock free from vestigial and from the other mutant characters known to be present. A preliminary test of the qualities of this purple stock was made by outcrossing a male to a wild female and carefully examining all F_2 flies (table 4). The F_2 showed only purple (150) and wild-type (300) flies as expected, but the ratio was 1:2 instead of 1:3. While this deviation was significant (4.1 times the probable error), it indicated a peculiarity of the wild parent rather than of the purple, and was not further regarded. The vestigial stock used was that from which purple itself was derived. It had been examined frequently and seemed to be clean.

TABLE 4

The F_2 offspring from the cross of a purple male to a wild female

1912, NOVEMBER 25	WILD TYPE		PURPLE	
	Females	Males	Females	Males
C178	118	81	35	32
C179	47	54	33	40
Total	300		150	

TABLE 5[1]

The B. C. offspring given by the F_1 wild-type sons, from the outcross of a purple vestigial male to a wild female, when back crossed to purple vestigial females

1913, JULY 7	NON-CROSSOVERS		CROSSOVERS	
	Purple vestigial	Wild type	Purple	Vestigial
DQ	62	52	0	0
DR	113	141	0	0
DS	131	96	0	0
DS′	34	28	0	0
DT	89	68	0	0
DT′	33	22	0	0
DU	90	112	0	0
Total	552	519	0	0

[1] This table and the next (table 6) were included by Morgan in his paper on "No crossing over in the male of Drosophila . . . .," Biol. Bull., April, 1914, pp. 200 and 201.

The question of crossing over in the male was the first point attacked. Complementary P_1 matings were made (June 13, 1913) by crossing purple vestigial to wild ('coupling') and by crossing purple to vestigial ('repulsion'). F_1 males from these matings were back crossed singly to purple vestigial females from the stock. The parents were in several cases transferred at the end of ten days to fresh culture bottles and second broods then raised.

The offspring from the 'coupling' experiment (table 5, 5 pairs, both broods) gave a total of 1071 flies, not one of which was a crossover, and the 'repulsion' experiment (table 6, three pairs, both broods) added 704 more (total 1775), not one of which was a crossover. Since these were back-cross experiments, there was no masking of results possible, and crossover gametes had every opportunity to reveal themselves had any been formed. Therefore, each fly recorded above is a true non-crossover. While the total absence of crossovers in these repetitions of the male test cannot prove that the apparent crossovers in the original test were not genuine crossovers, it added to the already large body of evidence which showed that they were aberrations from the normal condition.

TABLE 6

The B. C. offspring given by the F_1 wild-type sons, from the outcross of a purple male to a vestigial female, when back crossed to purple vestigial females

1913, JULY 7	NON-CROSSOVERS		CROSSOVERS	
	Purple	Vestigial	Purple vestigial	Wild type
DV	62	42	0	0
DV′	70	78	0	0
DW	61	53	0	0
DX	66	103	0	0
DX′	79	90	0	0
Total	346	358	0	0

The second point attacked was the amount of crossing over in the female between the loci purple and vestigial. F_1 daughters from the same two complemetery crosses that had furnished the material for the male tests just given were back crossed singly to purple vestigial males.

BALANCED INVIABILITY—COMPLEMENTARY CROSSES

The reason why both 'coupling' and 'repulsion' experiments were made is that by combining the two sets of data one can calculate a linkage value more nearly free from the errors due to disproportionate inviability of any class (Bridges, '15; Muller, '16). Within each back cross the inviability effects due to a given mutant form are largely neutralized. Since the inviable form occurs both as a crossover and as a non-crossover, both of these classes are lowered, but lowered proportionately, so that the linkage ratio remains practically undisturbed. This internal balancing holds less well for combinations of characters, for any given combination occurs in an experiment either as a crossover or as a non-crossover, but not as both, and should any combination have an inviability disproportionate to that of the component mutant forms, then the crossover value would be disturbed. The remedy for this condition is to balance the experiments in which a relatively invariable class occurs as

a crossover by an equal amount of data in which this same class is a non-crossover. It is often not convenient or possible to have complementary crosses of equal weight; but whatever is done in that direction, however little, is of advantage, and even a partially balanced result is to be preferred to one from only one type of cross. With improvements in culture methods, inviability effects have been very much reduced everywhere. Also with the great increase in the number of mutations, there is now provided an abundance of forms which show only negligible inviability. Our regular work utilizes only these viable forms, and except for very special purposes those mutants which show more than a slight inviability are avoided.

The first back crosses of purple vestigial had shown a marked inviability for vestigial and a slight inviability for purple. The new back crosses showed practically no inviability for purple and a very moderate amount for vestigial, but still enough to repay the added labor required by the balancing cross. As in the first back cross test of the female, the linkage shown was fairly strong. Since the linkage shown by second broods proved to be different from that of firsts, only first broods will be considered for the moment. The 'coupling' experiment (table 7) gave a total of 2839 first-brood flies, of which 305, or 10.7 per cent, were crossovers. The 'repulsion' first broods (table 8) gave a total of 2335 flies, of which 303, or 13 per cent, were crossovers. When the first-brood data from both these experiments are combined so that the inviability is balanced, the crossover value is 11.8 (table 9).

These two component crossover values differed slightly from each other and from the value (9.1) obtained in the original experiment. It may be questioned whether the difference in the crossover values was entirely due to inviability. Slight differences of this order, but many of them undoubtedly significant, are continually appearing in our work. Other known causes of linkage variation besides inviability are: differences in the age of parents (Bridges, '15), or of the temperatures at which the experiments are conducted (Plough, '17), or mutant 'crossover' genes (Sturtevant, Muller, and Bridges), and probably to several

TABLE 7

The B. C. offspring given by the F_1 wild-type daughters, from the outcross of a purple vestigial male to a wild female, when back crossed to purple vestigial males

1913, JULY 5	NON-CROSSOVERS		CROSSOVERS		PER CENT OF CROSSOVERS	CHANGE WITH AGE
	Purple vestigial	Wild type	Purple	Vestigial		
DA	178	202	16	16	7.8	
DA′	152	227	13	14	6.6	−1.2
DB	91	100	18	13	14.0	
DB′	69	104	12	8	10.3	−3.7
DC	165	150	17	19	10.3	
DC′	191	216	18	17	7.9	−2.4
DD	140	149	20	15	10.8	
DD′	116	122	9	4	5.2	−5.6
DE	191	214	20	19	9.0	
DE′	196	229	11	22	7.2	−1.8
DF	202	226	20	22	8.9	
DF′	197	228	25	20	9.6	+0.7
DG	105	158	17	17	11.4	
DG′	188	232	17	14	6.9	−4.5
DH	123	140	26	30	17.6	
DH′	129	179	11	20	9.1	−8.5
Firsts	1195	1339	154	151	10.7	
Seconds	1238	1539	116	119	7.8	−2.9

other internal and external factors not yet analyzed. The best that can be done in correction is to calculate mean values from as many experiments as possible where none of the recognized causes of variation are especially active and thus obtain a sort of composite picture of the 'normal' condition.

TABLE 8

The B. C. offspring given by the F_1 wild-type daughters, from the outcross of a purple male to a vestigial female, when back crossed to purple vestigial males

B.C.	NON-CROSSOVERS		CROSSOVERS		PER CENT OF CROSSOVERS	CHANGE WITH AGE
	Purple	Vestigial	Purple vestigial	Wild type		
DI	157	178	26	21	12.3	
DI′	200	165	12	14	6.7	−5.6
DJ	198	176	23	23	11.0	
DJ′	242	195	19	26	9.3	−1.7
DK	252	227	34	38	13.1	
DK′	198	178	26	20	10.9	−2.2
DM	205	158	27	32	14.0	
DM′	213	246	14	23	7.4	−6.6
DN	66	54	6	11	12.4	
DN′	66	64	4	7	7.8	−4.6
DO	189	172	30	32	14.6	
DO′	217	225	13	18	6.5	−8.1
Firsts	1067	965	146	157	13.0	
Seconds	1136	1073	88	108	8.1	−4.9

TABLE 9

Linkage of purple and vestigial with balanced inviability

	NON-CROSSOVERS	CROSSOVERS	TOTAL	PER CENT OF CROSSOVERS
Purple	1067	154		
Vestigial	965	151		
Purple vestigial	1195	146		
Wild type	1339	157		
Total	4566	608	5174	11.8

THE VARIATION OF CROSSING OVER WITH AGE

The reason for raising second broods in these experiments was to obtain more offspring from each female and thus secure a more trustworthy index of the genetic behavior of each individual. This practice was extended to all the work at this time, and was continued until a comparison of the crossover values of the first and second broods brought out a remarkable relation in the cases involving the second chromosome. There was found to be a change in the amount of crossing over so that both in the totals for each experiment and in a great majority of the individual cultures the crossover value had fallen significantly. Equally surprising was the fact that there was no such change in the case of the first chromosome, and this added another proof of the distinctness of our linkage groups, that is, of the individuality of the chromosomes involved. The first case in which this decrease for the second chromosome was clearly seen was that of the back cross tests of the purple vestigial linkage given in tables 7 and 8. Of the eight females whose tests are given in table 7 seven showed a decrease in the percentage of crossing over and only one (F) showed an increase, which, however, was smaller in amount than the smallest of the decreases. In the complementary case 'repulsion' (table 8) all six females showed a decided drop. The totals likewise reflected this same change; the decreases were 2.9 and 4.9 units, respectively. The crossover value calculated from the balanced second broods was 8, a decrease of 3.8 units, or, compared with the corresponding crossover value (11.8) from the balanced first broods, a 32 per cent decrease from the normal amount. Many other experiments have confirmed the fact of change in crossing-over frequency with the age of the mother, and some slight analysis has been made of the mechanism behind the results (Bridges '15).

THE LOCUS OF PURPLE—A TWO-POINT MAP

The repetition of the purple vestigial back crosses was not carried out until the summer of 1913; meanwhile considerable progress had been made with the mapping of the second chromo-

some. The test of the amount of crossing over in the female between the loci purple and vestigial (table 3) had given a crossover value of 9.1 units. The next crossover value to be worked out was that of black vestigial as about 20 units (Morgan, '12).

A THREE-POINT MAP

With these two values alone it was not possible to determine the relative order within the chromosome of the three loci involved; it was apparent that black was farther away from vestigial than from purple, but it could not be told whether it lay on the same or on the other side of vestigial from purple. This value was expected to be one of two values depending on the order of the genes; it should be an approximation to either the sum (20 + 9 = 29) or the difference (20 − 9 = 11) between the black vestigial and the purple vestigial values. To carry out a back-cross experiment for black and purple it was first necessary to make up the double recessive. No easy task was anticipated in this, for it has just become known that on account of no crossing over in the male no double recessive could be obtained in F_2, and in fact none was obtained (table 10). As expected, the F_2 ratio approximated 2:1:1:0. Three sorts of F_3 mass culture matings were made: black × black, purple × purple, and black × purple. Of these matings the last type is by far the most valuable, since in case one of the flies happened to come from a black purple crossover egg × a black sperm it would give some purple offspring when crossed to purple; and these inbred, would give the required black purples as a quarter

TABLE 10

P_1 mating, purple ♂ × black ♀; F_1 mating, wild-type ♀♀ and ♂♂

F_2, 1912, OCTOBER 24	WILD TYPE	BLACK	PURPLE	BLACK PURPLE
C68	248	137	136	0
C69	278	103	157	0
C70	158	60	78	0
Total	684	300	371	0

of the next generation. Likewise, if one of the purples had come from a crossover black purple egg the black × purple cross would produce some blacks that would give the required black purples upon inbreeding. If both the black and the purple chosen happened to have come from crossover eggs, then the double would be produced in F_3 directly. In case none of the parents proved to be from crossover gametes then at least the F_3 wild-type flies are equivalent to the F_1 and would save a generation in the repetition. The other two types of crosses would give a favorable result only if both parents happened to be from crossover eggs, in which case the double would appear among their progeny. It so happened that one of the black × black crosses gave a few black purples in F_3 directly, and from these a stock was made for use in back crossing. At the same time a P_1 mating of a black male to a purple female was started to furnish the required F_1 heterozygotes. A single test of the F_1 male showed, as expected, no crossing over whatever in the male (table 11).

Two back-cross tests of the female gave a total of 773 flies of which 38, or 4.9 per cent were crossovers (table 12). Of the two expected values, that of 30 is excluded entirely, and that of

TABLE 11

P_1 mating, purple ♂ × black ♀; B. C., F_1 ♂ × black purple ♀

B. C. OF MALE 1912, DECEMBER 14	NON-CROSSOVERS		CROSSOVERS MALE TEST	
	Black	Purple	Black purple	Wild type
II2	74	71	0	0

TABLE 12

F_1 mating, purple ♂ × black ♀; B. C., F_1 ♀ × black purple ♂

B. C. OF FEMALE 1912, DECEMBER 12	NON-CROSSOVERS		CROSSOVERS	
	Black	Purple	Black purple	Wild type
C174	320	339	13	18
II1	33	43	3	4
Total	353	382	16	22

10 is approximated, though not very closely. On this basis, the order of these genes is black purple vestigial, and not black vestigial purple.

A THREE-POINT BLACK CROSS, BLACK PURPLE CURVED, WITH BALANCED INVIABILITY

Most of the linkage experiments up to this time had involved only two loci, as the three just cited, namely, purple vestigial, black vestigial, and black purple. It was now realized that a more complex type of experiment involving all three loci at once would yield returns whose value far outweighed the greater labor entailed. Thus, a multiple back cross for black purple vestigial would give linkage data upon all three crossover values simultaneously, and these values would be strictly comparable, since there would be no possibility of discrepancies due to different conditions of culture or parentage. Accordingly, the simple black purple back cross was done on a scale only large enough to decide between two possible values and thus show what was the order of the three loci. A knowledge of this order is of great advantage in synthesizing the multiple recessive. It was found, as already stated, that black and vestigial are the two farthest apart and the mating was accordingly arranged so that a crossover anywhere within this whole distance would give the required triple form. That is, black purple and purple vestigial were mated together and the resulting purple offspring $\left(\frac{\text{b pr} +}{+ \text{ pr vg}}\right)$ were inbred. The F_2 black purples and purple vestigials were crossed together in several mass cultures, and in F_3 some triples occurred, showing that some of both kinds of F_2 flies used had come from crossovers eggs. A better method would have been to back cross the F_1 female by a black vestigial male. In this case every black vestigial crossover would be known to be of the composition $\left(\frac{\text{b pr vg}}{\text{b} + \text{vg}}\right)$, and these inbred would give the pure triple without the chance of failure that the method, actually used, ran. A stock of black purple vestigial was made from the triple recessives that hatched in F_3 (March, 1913).

In carrying out the triple back cross, the principle of balancing the inviability by complementary crosses was applied. To completely balance a three locus experiment required four types of crosses, so that every class may appear in each of the four crossover categories, namely, (0) non-crossovers, (1) crossovers in the first region, that between black and purple, (2) crossovers in the second region, that between purple and vestigial, and (1, 2) double crossovers, the simultaneous occurrences of crossing over in both regions. Thus, the cross of black purple vestigial by wild and the back cross of the F_1 wild-type daughters by the triple recessive male gave one of the four types of crosses (table 13). The other types of experiment carried out were black by purple vestigial (table 14), black vestigial by purple (table 15), and black purple by vestigial (table 16). In order

TABLE 13

P_1 mating, black purple vestigial ♂ × wild female; B. C. mating F_1 wild-type ♀ × black purple vestigial ♂

1914, JANUARY 9	b pr vg		b / pr vg		b pr / vg		b / pr / vg	
	Black purple vestigial	Wild type	Black	Purple vestigial	Black purple	vestigial	Black vestigial	Purple
II141	89	140	3	1	11	12	1	1
II142	118	137	3	3	15	9	—	—
II143	61	78	2	4	12	11	—	—
Total	268	355	8	8	38	32	1	1

TABLE 14

P_1, black × purple vestigial; B. C. test of F_1 ♀♀ singly

1914, MARCH 9	b pr vg		b / pr vg		b / pr vg		b / pr / vg	
88	114	96	10	10	7	15	1	1
103	92	81	15	5	12	16	1	0
104	99	98	11	15	8	17	0	0
116	97	66	2	12	14	13	0	1
116	164	77	6	15	12	19	2	0
Total	566	418	44	57	53	80	4	2

TABLE 15

P_1, black vestigial × purple; B. C. test of F_1 ♀♀ singly

1914, MARCH 10	b vg / pr		b pr / vg		b / pr		b pr vg	
101	85	126	10	7	23	15	1	0
102	137	133	13	13	16	23	0	0
112	67	68	7	7	12	8	0	1
113	92	137	13	8	21	9	1	1
Total...	381	464	43	35	72	55	2	2

TABLE 16

P_1, black × purple × vestigial; B. C., of F_1 ♀♀ singly

1914, OCTOBER 28	b pr / vg		b / vg pr		b pr / vg		b / pr vg	
654	130	152	10	5	14	10	0	0
670	124	111	10	8	11	14	1	1
671	137	138	15	6	12	30	2	2
672	131	154	7	10	18	12	0	1
673	162	151	11	7	12	13	2	0
674	159	162	8	7	16	24	0	2
Total...	843	868	61	43	83	103	5	6

that these four crosses should balance closely, the same number of cultures (six) was started in each case. A few of these cultures failed, and the total data in the separate experiments are consequently not in equal amounts. The balance is for this reason not perfect, though such partially balanced results are far better than an equal amount of data secured from only one of the four possible types of experiment. Additional cultures could have been raised until a balance was reached, and such a practice has been followed in other cases, for example, the vermilion sable forked case reported by Morgan and Bridges ('16). A summary of these complementary crosses appears in table 17, from which the following balanced crossover values are calculated: black purple 6.4, purple vestigial 10.8, and black vestigial 16.3.

TABLE 17

A summary of the four types of black purple vestigial black cross, with inviability balanced

COMBINATIONS	0	1	2	12	TOTAL
b pr vg	268 355	8 8	38 32	1 1	711
	623	16	70	2	
b	566 418	44 57	33 80	4 2	1224
pr vg	984	101	133	6	
b pr	843 868	61 43	83 103	5 6	2012
vg	1711	104	186	11	
b vg	381 464	43 35	72 55	2 2	1054
pr	845	78	127	4	
Total.....	4163	299	516	23	5001

COINCIDENCE

Another and very important advantage of these more complex crosses is that the process of double crossing over can be examined. Thus there were 23 double crossovers, or 0.46 per cent of the total flies. If the proportion of double crossovers were determined by chance alone the percentage should have been 6.4 per cent of 10.8 per cent or 0.69 per cent of the total. The observed per cent of coincident crossovers (0.46) is only 61 per cent of the theoretical per cent (0.69). This percentage, 61, is called the 'coincidence' for black purple vestigial. This index can be more conveniently calculated directly from the black-cross numbers as follows:

$$\frac{\text{No. doubles} \times \text{Total flies} \times 100}{\text{Total firsts} \times \text{Total seconds}} = \frac{23 \times 5001 \times 100}{322 \times 539} = 61.3$$

(Weinstein, '18)

THE RELATION BETWEEN COINCIDENCE AND MAP DISTANCE

The coincidence of 61 observed in this case is relatively very high. A coincidence under 5 is expected for cases in the first chromosome where similar map distances are involved. This higher coincidence may mean that for some reason the freedom of crossing over is much less in this region of the second chromosome than it is in the first chromosome. The 17.5 units of map distance between black and vestigial may correspond to as great a length of actual chromosome as is involved in cases in the first chromosome where the coincidence is the same but the map distances are nearly three times as great. On the other hand, instead of the higher coincidence being due to a lower 'coefficient of crossing over,' it may be due to a relatively short 'average internode.' The length of chromosome represented by a given map distance may be the same in the two regions compared, but in the second chromosome the mechanism of double crossing over may not require so long a section of chromosome between successive crossovers. If the average length of the internode was shorter because of this closer spacing of doubles, then a greater proportion of doubles would occur in the given region from black to vestigial, and coincidence would be correspondingly higher. However, the interest of these problems in double crossing over is out of all proportion to our progress in their solutions. It seems likely that it will be possible to derive a more satisfactory method of expressing these relations than is provided by the present formula; the new formulation must take account of separate factors analyzable in the process, and permit of their adequate representation. The conclusions based on the old formula must be regarded as provisional.

THE USE OF PURPLE IN MAPPING OTHER GENES, CURVED, STREAK, ETC.

The 'map' of the second chromosome began to be useful when the order and spacing for the three genes black purple and vestigial were roughly established by the determination of the third value, that for black purple (December, '12). The three

locus experiment just given provided more accurate measures of the map distances involved. The preparation of the multiple recessive and of the P_1 stocks delayed the completion of the experiment for nearly a year (January, '14). Meanwhile, these provisional locations were used as the basis for locating certain other genes more closely. The first of these was curved. Bridges and Sturtevant (Biol. Bull., '14) soon found (January, '13) that black and curved gave approximately 23 per cent of crossing over. The next point to be determined was the relation of curved and one of the other two mutations whose loci had been mapped. Both of these tests were used, since each offered advantages; the chief disadvantage was that vestigial interferes with the classification of curved, so that it is impossible to distinguish between the simple vestigial and the vestigial curved class.

The purple curved test was undertaken by Bridges, who prepared to run a three-point back cross involving black purple and curved. The first step was the synthesis of the purple curved double recessive. As soon as this was obtained it was turned over to W. S. Adkins, who ran a preliminary back-cross test of the simple purple curved crossing over, and found that there was about 18 per cent of crossing over. This enabled us to determine the relation of curved to the other three genes. The purple curved value of 18 showed that curved was closer to purple than to black (black curved = 23) and that purple and vestigial were therefore 'to the right' of black. Curved was further to the right than vestigial since black and vestigial gave only about 18 per cent of crossing over.

The vestigial curved distance was tested by Sturtevant, who found that there was about 8.5 per cent of crossing over. Because of the difficulty of classification already referred to, it was not thought worth while to run these tests on a large scale. However, vestigial is itself accurately mapped and is nearer to curved than purple is. These considerations are strong enough to warrant an extension of the vestigial curved tests which may furnish the main basis for the accurate mapping of curved.

Meanwhile, the purple curved test, while less satisfactory because of the longer interval with the attendant correction necessary for double crossing over, was more readily handled, and this led to a rapid accumulation of data on the purple curved crossover value. The black purple curved triple recessive was obtained (May, 1913), and the back cross itself carried out. Thirteen of the F_1 wild-type daughters form the cross of black purple curved to wild-type were tested by back crossing singly to males of the triple form. These same parents were, at the end of ten days, transferred to fresh culture bottles and second broods were raised. The details of the data of these cultures have already been published (Bridges, '15) and we need repeat here only the totals for the first broods (table 18).

TABLE 18

The total offspring of the first broods of the black purple curved × wild back ross (details published Jour. Exp. Zool., July 1913, p. 8)

	b pr c		b / pr c		b pr / c		b / pr / c		TOTAL	b pr VALUE	pr c VALUE	b c VALUE
August 24, 1913.....	1476	1577	96	74	339	330	19	23	3934	5.4	18.1	21.3

The second broods confirmed on a large scale the fact first brought to light in the purple vestigial back crosses (tables 7 and 8), that in the second chromosome the amount of crossing over changes with the age of the mother (Bridges, '15).

The numerical relations in the first broods of this experiment confirmed the position of curved as already mapped. A map of the second chromosome was constructed on the basis of the data then on hand (October, '13), and was as follows:

b	pr	vg	c
0.0	5.3	17.5	25.0

The black purple curved back cross was carried out in only one of the four possible ways, and is therefore unbalanced. However, the results showed that inviability was negligible. The little deviation from expectation can be attributed to curved, which appeared to the extent of 97 flies for every hundred expected.

'ALTERNATED' BACK CROSSES

In cases where only one type of back cross is to be made the poorest type is that in which all the mutant genes are in the same homologue as was the case in the black purple curved × wild experiment just cited. The flies having the most mutant characters are relatively the least viable, and this type of cross furnishes the highest proportion of such individuals. The best type is that known as 'alternated,' where the successive genes alternate between the two chromosomes $\frac{(\text{b} + \text{c})}{+ \text{pr} +}$ so that the maximum of evenness of distribution of characters is attained. It required double crossing over to put all the mutant characters in the same individual, and accordingly the 'alternated' experi-

TABLE 19

The partially balanced back-crosses involving streak, purple, and curved

COMBINATION	DATE	0	1	2	1, 2	TOTAL
$\frac{\text{Sk}}{\text{pr c}}$	1913 Nov. 6	435	247	127	69	878
$\frac{\text{Sk pr}}{\text{c}}$	1913 Dec. 18	496	254	117	62	929
Total.....		931	501	244	131	1807

ment gives a minimum number of the combination that is most inviable. This principle becomes still more important in more complex experiments, as, for example, $\frac{\text{S} + \text{b} + \text{c} + \text{sp}}{+ \text{d} + \text{pr} + \text{px} +}$.

The next mutant whose locus was mapped with reference to purple as a base was 'streak,' a dominant character which shows as a dark streak from the scutellum forward along the dorsal region of the thorax. The triple back-cross streak purple curved, which was made in two of the four possible ways, and is therefore partially balanced (table 19), showed that streak is far to the left of purple, that is, beyond black, and in the opposite direction from vestigial and curved. The streak purple

crossover value was 35, which showed that streak is so far to the left of purple that only an approximate calculation of its position could be made from the data. In a region of such length the correction to be supplied because of double crossing over is quite large and correspondingly inexact. On the basis of data that have since become available it appears that there is about 37.3 per cent of crossing over between streak and purple. Purple has played an important rôle in the mapping of several other genes, the details of which will appear in accounts of these mutations.

A SUMMARY OF THE LINKAGE DATA INVOLVING PURPLE

Besides the data reported in this paper, there are available data from four other principal papers—Bridges' study of age variation in crossing over (Jour. Exp. Zoöl., '15), Muller's study of crossing over by means of the progeny test (Am. Nat., '16), Plough's study of temperature variations in crossing over, and the data given in various sections of a Carnegie publication by Bridges and Morgan that is soon to appear. Table 20 gives the totals for all these data collected according to two loci calculations. The black purple crossover value of 6.2 based on 48,931 flies places the locus of purple at 6.2 units to the right of black, or at 52.7 when referred to star as the zero point.

TABLE 20

A summary of the purple crossover data

LOCI	TOTAL	CROSSOVERS	PER CENT
Star purple	8,155	3,561	43.7
Streak purple	2,665	883	33.1
Dachs purple	1,489	293	19.7
Black purple	48,931	3,026	6.2
Purple vestigial	13,601	1,609	11.8
Purple curved	51,136	10,205	19.9
Purple plexus	344	164	47.7
Purple arc	2,625	1,066	40.6
Purple speck	11,985	5,474	45.7
Purple balloon	462	218	47.2

SPECIAL PROBLEMS INVOLVING PURPLE—AGE VARIATIONS, COINCIDENCE, TEMPERATURE VARIATIONS, CROSSOVER MUTATIONS PROGENY TEST FOR CROSSING OVER

We have already seen how the study of the age variation in crossing over for the second chromosome began with the purple vestigial back cross (p. 283) and was continued and confirmed by the black purple curved triple back cross (p. 294). Some of the early data suggested that the drop in the second broods was followed by a recovery and perhaps even by a rise in later broods (Bridges' '15).

To gain further light on the course of the variation throughout the life of the fly, a special and extensive experiment was continued through four broods. The entire length of the chromosome was covered by the loci chosen $\left(\frac{S\ +\ +\ +}{+\ pr\ c\ sp}\right)$.

This experiment showed the normal crossover values for the first broods, the usual drop for the second broods, and a slight continued drop for the third and fourth broods. However, the experiment proved inconclusive because of two ill adaptations: the chromosome distances involved were so long (e.g., S pr = 52.7) that real changes could be concealed by a concomitant change in double crossing over, and further because the ten-day broods gave only four points on the curve of age variation, each point representing only the net change for a ten-day period, while the real underlying curve may have changed its course so that an unknown part of each ten-day period may have been a fall and the rest a rise.

The original black purple curved experiment had avoided one of these difficulties in that the black purple distance is so short that there is probably no double crossing over whatever within it. The second difficulty was met by Plough in his similar studies on the temperature variation of linkage by transferring his parents every two days instead of every ten (Plough, '17). As one of his control experiments Plough ran a black purple curved back cross of thirteen pairs, transferring each pair to a fresh culture tube every two days as long as the female lived (table 14, Plough, '17). The plotted curve of the percentages

of crossing over between black and purple shows an initial high value (8 per cent) which during the first nine days falls rapidly at first and then more slowly to a low value (5 per cent), which is maintained with little change to about the sixteenth day. A sharp rise then sets in which reaches its maximum (8 per cent) at about the twenty-first day. The succeeding fall is again slow, reaching its minimum (3.5 per cent) at about the thirtieth day. Beyond this point the curve again rises slightly, but the data were too few to be significant beyond about the twenty-fifth day. While there was some variation in the amount and rapidity of these changes in the various individual curves, all showed the same typical rhythm, which must be the expression of fundamental physiological changes in the development of the female. It seems possible and probable that these successive falls and rises are not effects of a single continuously varying physiological process, but are rather to be explained as separate phenomena caused by the lapse of certain conditions and the subsequent onset of new causes. These changes may therefore be really discontinuous and the rhythmic curve only a succession of independent but overlapping variations.

The most interesting feature of the age variation is the bearing it has on the problem of double crossing over and the underlying problems of the nature of crossing over. The more consideration that has been given to this problem of double crossing over in relation to chromosome and to map distances, the more involved it has appeared, so that no evidence upon these points can be neglected. The special value of such cases as that of age variation is that they enable one to compare two different conditions, but with the elimination of one important variable; for, the actual chromosome distance between two given loci is maintained constant, so that any variations that occur in map distance, coincidence, etc., must be due to variations in one or more of the other factors. This relation was discussed in connection with the original two-brood black purple curved experiment (Bridges, '15), and it was pointed out that the rise in coincidence concomitant with the fall in crossing over meant that the internode length had changed—that the two

crossovers of a double no longer included the same average length of chromosome, but were including a greater length. The coincidences of the $\frac{S \; + \; + \; +}{+ \; pr \; c \; sp}$ case support this view, but in neither case are the data conclusive. A calculation of the coincidences shown by Plough's black purple curved two-day tube cultures has provided data considerably more satisfactory, though still subject to a high probable error. On the basis of all the data, it is probable not only that coincidence varies with age, but that the curve of age variation in coincidence is roughly the mirror image of the curve of age variation in crossing over. While it seems probable that at least part of the explanation of the age variation both in crossing over and in coincidence has been found in an internode variation as suggested, yet in any case there is provided evidence of a common cause that should repay further analysis.

A second problem involving purple and very closely allied to the age variation in conception, material, methods, and bearing, is that of the temperature variation described by Plough ('17). Since the genic constitution of a female showing the age variation is constant throughout the course of this variation, the immediate causes of the variations must be regarded as environmental differences arising through rhythmic changes in the physiological processes of nutrition and development. While the crossing-over variations due to age and to specific genes were affected through environmental changes arising internally, they suggested the possibility that similar variations might be initiated by environmental changes arising externally. Plough found that exposure to abnormally high or low temperature actually did produce linkage changes even more extreme than those due to age changes. Black purple curved back-cross cultures were raised at various temperatures from 9° to 32°C. When the black purple crossover values were plotted, it was seen that at a low temperature (9°), crossing over is very free (14 per cent) and becomes even more free at 13° (18 per cent). The amount of crossing over then falls away very rapidly, and at 18° has nearly returned to the normal value. The normal

value is maintained to about 27°, or throughout the whole range of 'room' temperature at which the breeding work is ordinarily conducted. At 29° the crossing over is slightly freer than normal, but between 29° and 31° the amount of crossing over nearly trebles (18.2). This extraordinarily sharp and extensive rise is followed by a slight fall at 32° (15.4 per cent). Above this temperature, and below 9°C. it was found that the flies either died or produced too few offspring to be workable. It seems probable that here also these two sharply marked maxima separated by a long interval of no or slight change may represent two distinct phenomena.

When the coincidences are calculated for these various temperatures, it is seen that the curve of temperature variation of coincidence is a slightly rising but practically straight line cutting alike through both of the maxima and the normal interval. When the variations in crossing over due to exposure to abnormal temperature are followed through the life of the fly by means of tube counts, characteristic rises and falls appear on the curve which expresses this relation. When a coincidence curve is calculated from these same data, it is seen that it cuts through the rises and falls due to temperature exactly in the same way as it did in the temperature and coincidence curves constructed from experiments at diverse temperatures. This is a significant difference from the relation previously observed in the age variations, and would seem to indicate that the age and temperature variations were accomplished by different mechanisms—by effects upon different physiological factors. Those double crossovers that do occur have the same distribution along the chromosome at all temperatures, which shows that the method of handling the chromosomes is unchanged. In accordance with the analysis already given (p. 298) the cause of the temperature variations in crossing over is to be sought rather in variation in the coefficient of crossing over—in the crossing over capacity of the chromosome itself because of some variation in its structure or framework.

The conclusion just drawn from the failure of the temperature variation to affect the coincidence, namely, that the change in

the amount of crossing over is probably due to a change in the physical properties of the chromosome substance, has an important bearing on the question of the stage at which crossing over itself occurs, as follows: From a study of the time taken for the effects of exposure to abnormal temperature to become manifest or to disappear, Plough concluded that the effect was produced at one stage only in the development of the ovary, and that eggs which have not arrived at or have passed this critical stage are incapable of registering any temperature variation. It was next argued that this critical stage is that at which crossing over itself normally occurs. It is certain that crossing over does not take place before this critical stage is reached, but it does not follow that it might not occur at some stage between this and the maturation divisions; that is, at any later stage during the growth period. At the critical stage one of the factors which modifies the frequency of the crossing over becomes fixed, but the crossing over itself may occur later. As a crude analogy, the process of crossing over might be likened to a machine—say, a sawmill. The rate at which boards are sawn depends, other factors remaining constant, upon the toughness of the log fed against the saws, which toughness is a physical property of the log fixed long previously.

The coincidence analysis indicates that the setting of the crossing-over machine has not been altered, but that the chromosome at a specific sensitive stage in its fabrication has been modified in one of its properties—its toughness, let us say—so that when it ultimately undergoes crossing over the output is different.

It is quite possible that the crossing over follows immediately after the determination of this property; indeed, from other lines of evidence it seems probable that crossing over occurs at a thin-thread stage, or at least that the characteristic transjunction is accomplished at a leptotene stage, such as occurs only in the early growth period. But such a conception does not exclude the possibility that the crossing over occurs at a four-strand stage, as is indicated by still other lines of evidence. To call such a 'thin-thread, four-strand, early-growth stage,'

conception of crossing over 'chiasmatype' would be misleading, since the term is usually understood as applying to a 'thick-thread, four-strand late growth-stage' condition. The term 'tetraleptotenic' might be used for this type of crossing over to distinguish it from both the 'dileptotenic' and the 'chiasmatype' hypotheses.

Plough's evidence that the critical stage and crossing over occur after most or probably all of the oögonial divisions have been completed effectually disproves the reduplication hypothesis of crossing over as far as any application to Drosophila is concerned, for the number of divisions required by that hypothesis is not available.

Purple has been extensively used in two other important studies on crossing over—that of Sturtevant upon inherited crossing-over variations (in press), and that of Muller (Muller, '16) in his progeny test of a multiple heterozygote in studying crossing over.

SUMMARY AND VALUATION

Because of its excellent characteristics, purple has proved especially useful as a tool in the general work on the second chromosome. In viability, fertility, productivity, and in the many details of 'habit'—ease of handling, activity, time of hatching, length of life, etc.—purple measures well up to the standard of the wild fly. It is separable from the wild type with certainty and rapidity. A difficulty in classification may arise from the occurrence in the same culture of a similar eye color—a 'mimic' or 'pseudopurple'—either by mutation or by introduction. However, the presence of a mimic is generally easily recognized and such difficulties in classification are only temporary.

The usefulness of purple has not been restricted by 'masking effects': until very recently there has been no other readily workable second chromosome eye color so similar to purple in appearance as to prevent the use of both in the same experiment without confusion between them. Purple is not so dilute that it would interfere with the classification of the eye colors, as

does 'white' in the X chromosome; nor, conversely, is there any other second chromosome eye color so dilute (or morphological change so extreme) as to interfere with the classification of purple on flies possessing both characters.

The recessiveness of purple seems to be complete and constant, so that there is no chance of confusion between it and the heterozygote.

The locus of purple on the basis of very extensive data is 6.2 units to the right of black, or, referred to star as a base, at 52.7. Purple is therefore practically at the middle of the second chromosome, and is thus useful in combination with mutations whose genes are located anywhere throughout the chromosome. Its closeness to black, the locus which is the primary base in the mapping of the second chromosome and another of the very best characters, furnishes a working distance which is short enough to exclude double crossing over and long enough to avoid the excessive probable errors incident to very small percentages of crossovers. Outside this black purple section the second chromosome is as yet mostly mapped in distances too great—or too small—to handle satisfactorily in special tests. Furthermore, it appears that this purple region is peculiarly sensitive, as is proved by its exceptionally high double crossing over (this paper) by its greater disturbance by age (Bridges, '15; Plough, '17) and by its unique reaction to genetic variations in crossing over (Sturtevant, in press). The explanation of this sensitiveness is probably that this region is actually near the middle of the chromosome with the spindle fiber attachment, and that this middle region is the last part to undergo synapsis.

The number of subjects in the genetics of Drosophila toward whose early and continued development purple has contributed is surprisingly large.

In the field of mutation it gave with vermilion the first case in which 'intensification' or 'disproportionate modification' was recognized and made use of. It was the first of the class of 'dark' eye-color mutations. It has been one of the most popular models in Drosophila for 'mimic' mutations. The most striking 'epidemic of mutation' or 'mutating period' was that inaugurated by purple.

In the early experiments involving purple several other mutations arose, probably the most interesting of which was 'extra bristles,' which led to the study made by MacDowell on the effect of selection on bristle number.

In the attack upon the problem of 'inviability' purple entered into the first experiment planned to include the balancing of inviability by complementary crosses. This practice was extended to involve three locus experiments in the balancing of the black purple vestigial back cross. The inviability of vestigial met with in the early purple vestigial crosses seems not to have been due primarily to 'prematuration,' 'repugnance,' or autosomal lethals, but probably to culture conditions, as shown by Carver (unpublished).

In the development of autosomal linkage, purple was involved in the first coupling F_2, back-cross test of crossing over in the male, and likewise in the female. One of these back-cross tests of the male gave a few crossovers which prevented a clear conception of no crossing over in the male. The back-cross tests of the female gave the first 'two-point' autosomal map, purple vestigial. The first autosomal 'three-point' map was black purple vestigial, completed by the determination of the black purple crossover value.

With that most fascinating and difficult subject—the analysis of the relation between the physical chromosome and the process of crossing over—purple has been intimately connected. The relatively high coincidences obtained in the cases of black purple curved and black purple vestigial soon showed that this relationship in the purple region of the second chromosome is different from the relationship for sections of like map distance in the compared regions of the first chromosome. An expansion of this comparative study should aid in arriving at the cause of the differences.

The change in coincidence accompanying the age variation in crossing over in the case of black purple curved (Bridges, '15) led to the tentative conclusion that both changes were mainly due to a lengthening of the average length of the section of chromosome between coincident crossovers, rather than to a

changes in the freedom of crossing over. Certain other experiments, notably $\frac{S \; + \; + \; +}{+ \; pr \; c \; sp}$, which give information on the age and coincidence changes, have given results that agree better with the first interpretation, though they do not exclude the alternative. The clearest evidence pointing to an internode change is derived from the age-variation experiment made by Plough as a control for his temperature-change cultures ($\frac{b \; pr \; c}{+ \; + \; +}$ B. C., 22°, 2 day interval tube control). In this experiment the curve for variation in coincidence was the mirror image of the curve of variation in age. The curve of coincidence corresponding to the curve of temperature variation found by Plough seems to be a straight line cutting through the rises and falls of the temperature curve and independent of them. This suggests that the temperature variation is due to a change in a different physiological factor than that involved in the age variation, and that probably it is due to a modification of the coefficient of crossing over of the chromosome itself.

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Resumido por el autor, Edward C. Day.

La fisiología del sistema nervioso de los tunicados.

I. La relación del ganglio nervioso con las reacciones sensoriales.

Los bordes de los sifones de Ascidia mentula son mas sensibles a la acción de los estímulos tactiles, vibratorios y químicos que los de otras especies. La reacción sensorial es local cuando el estímulo tactil es muy débil, pero bajo la acción de uno mas intenso ambos sifones responden de un modo coordinado transcurriendo un periodo latente, que dura próximamente medio segundo, durante el paso del impulso nervioso desde un sifón al otro. La dirección de este impulso es reversible. Ascidia mentula es también sensible a los efectos del HCl, NaCl, NaOH y la quinina, pero no a los de la solución de azúcar de caña al 50 por ciento; es también muy sensible a los efectos de las vibraciones de la habitación en que esté colocada, pero por completo indiferente a la luz solar. Los sifones amputados retienen la sensibilidad durante cinco a seis dias. Los individuos desprovistos de sifones los regeneran aun cuando se haya separado el ganglio con uno de ellos. La extirpación del ganglio interrumpe la coordinación, disminuyendo el tono muscular y la sensibilidad. El tejido ganglionar se regenera, estableciéndose la coordinación al cabo de cuatro a seis semanas. Ascidia atra es semejante a Ascidia mentula en estructura y reacciones sensitivas. En oposición, a los tunicados mencionados, Ciona intestinalis es decididamente sensible a la acción de un rayo solar y el área sensible está localizada alrededor del ganglio. Los sifones de Ciona separados del cuerpo exhiben contracciones automáticas rítmicas.

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THE PHYSIOLOGY OF THE NERVOUS SYSTEM OF THE TUNICATE

I. THE RELATION OF THE NERVE GANGLION TO SENSORY RESPONSES

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FIVE FIGURES

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INTRODUCTION

When one beholds a solitary ascidian in normal surroundings, one comes to the conclusion that such an organism needs no complicated nervous system to cope with the exigencies of its environment. Unless the observer disturbs the ascidian by stamping on the sand, he may watch it for a considerable length of time without detecting any movements which would indicate it to be an animate object. From time to time, however, it is

seized with convulsions. The attacks, though violent, are brief, and soon the animal is as erect and motionless as before.

These sudden contractions at irregular intervals are the only vigorous muscular movements which the tunicate makes. They are performed in order to clear the pharyngeal sac of foreign particles, or to expel faeces, sperm, and mature ova from the atrial chamber. When the animal is strongly stimulated around either siphon, this characteristic vomiting reaction occurs. Stamping on the sand or clapping two stones under water will call forth a similar response. The lobes of either siphon are capable, however, of individual response and react to feeble stimulation with a local curling in of the lobe involved; a stronger stimulus causes puckering of the other lobes as well, while a still more vigorous application of the stimulus elicits an almost simultaneous closure of both siphons, the one stimulated being usually the first to respond.

This response of the siphons being an easily observed reaction, it served as a good indicator of the normal functioning of the nervous system. The circlet of tentacles just inside the aperture of the oral siphon which forms in some species a hairy strainer to prevent too large bodies from being swept into the pharynx with the ingoing current, could likewise be used as a reaction indicator, though with less success.

The lip-lobe reaction was by far the most delicate of the two, and through it the general irritability of the animal and the conductivity of the ganglion could be studied under varied experimental conditions. Through this reaction the relation of the central nervous system to the sense organs and musculature could be fairly well ascertained, but as an index of the relation of the ganglion to the activity of the visceral organs this reaction was useless.

While a few preliminary studies were made upon Ascidia atra Lesueur, a species found on the coral reefs of Bermuda, most of the investigations were carried out on Ascidia mentula, Ciona intestinalis, and Ascidia mammillata, but chiefly on Ascidia mentula, at the Naples Zoological Station.

My interest was first aroused in the subject of tunicate reactions during a zoological expedition from Harvard University to the Bermudas in 1910, and it led to later investigations carried out in 1913 at Naples. For the opportunity of enjoying the facilities of the Naples Station I am indebted, on the one hand, to Harvard University for a traveling fellowship, and on the other, to the Smithsonian Institution for the use of its research table there. I wish here to express my thanks to the various members of the Naples staff for their services and many kindnesses during my sojourn.

In how far Ascidia atra differs from Ascidia mentula in the finer details I cannot say, but in the gross anatomy they are very similar, although in color the former is bluish black and the latter a milky white. Both are from 4 to 6 inches in length, have a smooth outer tunic unornamented with protuberances, hairs, or other local modifications. The margins of incurrent and excurrent siphons of both species are lobed. The only record of the number of lobes which I have for Ascidia atra is a general statement that the number on the incurrent siphon ranges from 7 to 11, while that for the excurrent is 5 to 6. Hecht ('18) says 8 on the oral and 6 on the atrial. For Ascidia mentula the average number for the former is 9 and for the latter 6. The interlobular margins of both siphons of Ascidia mentula are edged with red pigment, while the lobes themselves are, as a rule, uncolored. Just proximal to these red edges are situated isolated pigment spots; a single one usually between each lobe on the oral siphon, and two or more in a cluster between each lobe on the aboral siphon. Occasionally an animal was brought to the laboratory which was totally brick red. Ascidia atra, on the other hand, is a velvety bluish black all over, due to the presence of pigment in the outer tunic. I never ran across one, however, where pigment was restricted to only the margins of the siphons. Ascidia mammillata, like Ascidia mentula, is whitish except for the pigment spots, in this case black, around the incurrent and excurrent apertures. Ciona intestinalis has red pigment spots on the margins of the siphons. Mention is made of these details of pigmentation because they

will later be referred to in connection with the sensitivity of the animals to light.

The procedure of experimentation was first to study the activities of the normal, undisturbed animal under as natural conditions as possible; second, to record its reactions to stimulations of various kinds, and, third, to discover the effect of various operative procedures such as incisions, amputations of siphons, and extirpation of the ganglion, upon the sensory reactions and the beat of the heart. Studies made upon the heart-beat will be given in a subsequent paper.

TACTILE REACTIONS OF NORMAL ACIDIA MENTULA

A. Observations on unstimulated individuals

By the term unstimulated is meant cases in which I introduced no stimulating agent myself, although the animals were never entirely free from disturbing influences.

The animals remain motionless for long intervals with both siphons wide open when they are in their natural environment of shore-water. I have observed the same thing in the case of animals attached to the walls of large cement tanks in the Naples Aquarium, where natural conditions have been duplicated as nearly as possible. In the laboratory, although the tanks were large and provided with running sea-water, the animals in them were not entirely insulated from disturbing vibrations in the room.

A protocol was kept of continuous observations on two specimens of Ascidia mentula for a period of about two hours and the time recorded for every closure of both incurrent and excurrent siphons. The animals were kept singly in large glass jars furnished with running sea-water. Both animals were very responsive to vibratory disturbances and closed their siphons whenever a door shut or somebody walked across the floor. Hecht ('18) finds Ascidia atra also extremely sensitive to vibrations in the room. The response consisted simply of a puckering of the marginal lobes of the siphons; seldom did the whole body contract. When the room was quiet for a length of time, for one

of the animals no reactions were observed, while for the other frequent contractions were noticed, especially of the atrial siphon. This siphon proved to contain one or two parasitic crustacea, 3 to 4 mm. long, partially embedded in the siphon near its tip, and whenever they kicked up a rumpus the siphon closed, and the closing of this siphon often induced closure of the other. The parasitized atrial siphon being in a quasi continuous state of irritation responded more readily to the vibrations in the room than did the unexcited siphon. Sometimes small crabs were found living symbiotically in the pharyngeal sacs of the tunicates.

B. Responses to tactile stimulation

To penciling with a bristle the siphons give local responses, thus only the lip-lobe on the stimulated side of the siphon puckers in, provided that the stimulus is feeble; to a stronger stimulus, however, all lobes of the stimulated siphon respond, and if the penciling be of sufficient strength, the closure of the incurrent siphon is followed by closure of the excurrent siphon as well.

The base and body of the animal are insensitive to penciling, while the necks of the siphons are slightly sensitive, though less so than the lip-lobes. The margins of the two siphons are therefore the most sensitive areas of the entire body. As to relative responsiveness, little difference could be discovered between oral and aboral siphons; in one or two cases the aboral or excurrent siphon was the more sensitive of the two.

EFFECT OF OPERATIONS UPON THE TACTILE RESPONSE

A. Slitting the siphons

When the oral siphon was slit lengthwise for half an inch, cleaving it in two parts, and the lobes of one side were stimulated, there was a response first in that half, then after a second's latent period a response in the other half. A reversal of the

sequence occurred by stimulating the other half first. Thus the wave of stimulation could travel in either direction around the cut. No effort was made to determine whether this stimulation wave was of the nature of a nerve impulse, or whether it was simply a wave of muscular contraction which depends for its completion upon the continuity of the muscle tissues; nor further, whether the length of the latent period was dependent upon the intensity of the stimulus to any degree, or in how far it was affected by the depth of the incision.

B. Amputation of siphons

When an Ascidia mentula was narcotized with cocain, the oral siphon amputated below the circlet of oral tentacles, and both the animal and the amputated piece were put into fresh sea-water and allowed to recover, the aboral siphon showed a return of sensitivity in a little over an hour while the amputated siphon did not regain sensitivity until two or three days after the operation. When the operation was performed without first narcotizing, recovery of the amputated piece took place in twelve to fourteen hours, whether it was the oral or aboral siphon.

Similar operations on the siphons of Ciona intestinalis revealed a greater recuperative power than that of Ascidia mentula, amputated siphons recovering sensitivity twenty-five minutes after the operation.

The siphons possess, therefore, an irritability which is independent of any connection with the nerve ganglion. The threshold of stimulation lies higher for amputated siphons than for the intact siphons, it requiring a stronger stimulation to elicit a response. This diminution in sensitivity was probably occasioned in part by the insufficient blood supply due to the operation, with a consequent lack of oxygen and an accumulation of catabolic products, and in part by the interruption of the nerve reflex. The presence or absence of the rings of oral tentacles made no obvious difference in the general responsiveness of the amputated oral siphon.

Not only did these amputated siphons exhibit independent irritability, but in the case of Ciona, an automatic rhythm was also manifested by the severed oral siphons. A siphon which had been cut off distal to the circlet of oral tentacles was observed two days after the operation to execute a series of rhythmical contractions which consisted of the periodic curling of the lip-lobes either inward or outward and the occasional constriction of the neck region. There was no special sequence in which the several lobes took part in the contraction, for a contraction would start on one side and be followed by a puckering, sometimes of the opposite side, sometimes of an adjacent part. The proximal portion of the amputated piece, i.e., the region of the cut margin, played no rôle in these contractions, the rhythmical movements being restricted to the oral margin and to the directly underlying portion of the neck. In fact, when the proximal part was stimulated, the response occurred not in the proximal end, but in the distal after a short latent period, the impulse having traveled from the less irritable region of stimulation to the more irritable region of the sensitive lobes before producing a response. No attempt was made to analyze the underlying phenomena of this response or to seek an explanation of the rhythmicity. No such rhythm was observed for amputated siphons of Ascidia mentula. As it has frequently been demonstrated that muscle tissue may be thrown into rhythmical contractions by the presence of various salts in the water, this rhythmical contraction in the Ciona siphon might have been due to the automatic response of either the muscle or the nerve tissue to the stimulating effect of the sea-water on the cut surfaces. Whether sensory cells are present in the siphon regions or not I have made no histological examination to ascertain, but nerve fibers can be seen extending out into the lip-lobes.

Tunicates whose siphons had been amputated regenerated new ones in the course of about three weeks, but amputated pieces lived only five or six days and died without any sign of beginning to regenerate a new body.

The three following protocols will give the history of such an operation with its subsequent effects both on the amputated siphons and on the desiphonated body:

Protocol 1. Ascidia mentula nos. 10, 11, and 12. April 17, 1913. Narcotized all three animals with cocaine in sea-water and cut off the tips of the siphons at XX′ on the oral or incurrent siphon (distal to oral tentacles) and at YY′ on the aboral or excurrent siphon (fig. 1). Returned animals to running sea-water.

April 18. Amputated pieces A and C are sensitive to tactile stimulation (B pieces not tested).

April 19. A and C respond to tactile stimulation, but not to tapping on the jar containing them. One of the bodies B is sensitive to the tapping and responds by ejecting water from the siphons. Pharyngeal sacs have fallen away from the cut surfaces of the siphons.

April 23. A and C pieces are dead. (The supply of running water had got accidentally shut off.) The body pieces, B, are alive and appear to be regenerating new tips.

May 9. New siphons have been formed on all three animals. These lie within the old cut tips and appear to have been developed from the pharyngeal sac. Distinct lip-lobes and interlobular pigment spots are visible on most of them: 7 to 8 lobes on 10C, 8 on 11A, 6 on 11C, 5 on 12A; on 10A and 12C the siphons are too puckered up to make out the number.

Protocol 2. Ascidia m. no. 5. April 12, 1913. Cocainized the animal (400 cc. sea-water + 1 cc. 5 per cent cocain), and amputated oral siphon below the circlet of oral tentacles at XX′ (fig. 2). Procedure was as follows:

5:10 P.M. Animal placed in cocain solution.

6:10 P.M. Benumbed; operated on, rinsed in fresh sea-water and returned to running sea-water.

6:45 P.M. Excurrent siphon sensitive to tactile stimulation; amputated incurrent siphon A, unresponsive.

April 15. A is responsive around marginal lobes; tentacles not. Lobes pucker in in response to tactile stimulation and to rap on substratum. B is very sensitive both on cut surface and on excurrent siphon.

April 16. A and B respond to tap on jar.

April 17. B responds to tap on jar; A, too, if tap is strong.

April 19. B responds to tap on jar; A no longer.

May 9. B has regenerated a new incurrent siphon with six small lip-lobes and a new ring of pharyngeal tentacles. New siphon puckers shut upon tapping the jar.

Protocol 3. Ascidia mentula no. 17. May 10. Incurrent siphon amputated to include the nerve ganglion. (Since the object of this experiment was to study the effects of the operation on the heart beat, no attention was paid to the amputated piece.)

June 3. A new incurrent siphon and ganglion have regenerated; six lobes to the siphon (fig. 3).

That the nerve ganglion is quite dispensable to the response of the siphons to stimulation is evident from the foregoing results. Amputating the siphons, however, involved cutting off the blood-supply from the severed part, and the effect of breaking all connections with the ganglion was so complicated with interrupting the circulation that no safe conclusions could be drawn with regard to the nervous control exerted by the ganglion. By removing the ganglion, therefore, without at the same time injuring the circulation, its relation to the siphonal responses could be separately determined.

C. Extirpation of the ganglion

Four Ascidiae mentulae were operated upon by excising the ganglia, and they lived long enough for the ganglia to regenerate —a period of about one month.

Immediately following the operation on two of the animals, the oral siphons began to open, but their irritability to stimulation was greatly reduced. Since the operation was performed without the use of a narcotic, this diminution of sensitiveness was probably due to shock. With the lapse of a few days irritability gradually increased, but it never attained the level of the normal animal until towards the end of the month when the nervous tissue had regenerated. Thus, although the ascidians responded to penciling and to tapping on the jar in which they lay, a greater strength of stimulus was required; the animals were no longer disturbed by those extraneous vibrations from the closing of doors and the treading of feet which had produced responses prior to the operation.

Another animal which had had the ganglion destroyed by painting it with nitric acid, also opened its oral siphon immediately (1 minute) after the operation, but kept its aboral siphon closed for some time afterwards. Three days later, both siphons were open and responded to tactile stimulation, but the responses were of an inferior order.

Two marked changes in the reactions of the animals were produced by these operations on the ganglia: 1) a decrease in

general sensitiveness and, 2) a complete break in the coördination of the siphons. While each siphon was capable of responding when stimulated directly, it did not join in the response when the other siphon was stimulated, as was the case before the operation. This state of incoördination lasted until the ganglionic tissue regenerated, about four to five weeks later.

A brief history of a single case will illustrate the course of events following an operation on the ganglion.

Protocol 4. Ascidia mentula no. 2. April 12. Animal removed from water and ganglion extirpated. Oral siphon opened immediately after operation and before animal was returned to the water; aboral siphon remained closed.

April 15. Siphons both responsive to tactile stimulation with a bristle and to tapping on table or jar with a scalpel. No coördination between the two. The tentacles are also sensitive to penciling, and when they are touched the oral siphon closes.

April 26. Siphons both responsive to stimulation. No coördination.

May 5. Faint signs of recoördination between the siphons.

May 9. Coördination of siphons definitely reestablished. Out of ten trials in which the oral siphon was stimulated with a camel's-hair brush, the oral siphon responded ten times and the aboral seven, the sequence of contraction being from oral to aboral with a latent period of about one-half second between the two responses. When the aboral was stimulated, out of ten trials the oral siphon responded five times to its ten, the sequence being aboral to oral. The regenerated ganglion can be recognized as a small whitish body lying in the region of the original one.

The other three animals which had been operated upon at the same time as the one whose history has been given above, had also regenerated their ganglia, and, upon being tested, showed that the coördination of the siphons had likewise been reestablished. The fifth ascidian, whose ganglion had been destroyed with nitric acid, died a week after the operation. Up to that time, however, the siphons retained their independent sensitivity, but no coördination existed between them.

Two weeks earlier, but three weeks after the removal of the ganglia, none of the four ascidians exhibited any signs of coordination, although the siphons responded independently to direct stimulation. At the end of five weeks, however, the stimulation of one siphon brought about not only the closure of

that siphon, but also the closure of the other siphon as well. The unstimulated siphon did not always respond, owing apparently to the exhausted condition of the ganglion which seemed to fatigue quickly with too frequent an application of the stimulus. As no method was employed either for measuring the strength of the stimulus or for graphically recording the response, no exact comparison could be made between the original normal responses and those subsequent to regeneration of the ganglion. The sensitivity of the animal as a whole, however, did not seem so great after the restoration of ganglionic tissues as before the ganglion had been extirpated, for although the siphons responded readily to feeble penciling with a camel's-hair brush, they did not respond to vibratory disturbances, such as the shutting of doors and the treading of feet. This inability to respond to vibrations may find its explanation in the ease with which the ganglion is fatigued, because, even though the ganglion were composed of nervous tissue of a higher degree of sensitivity than before, the depressing effect of frequent vibratory stimulations would prevent the state of irritability from attaining to a maximum. The latent period between the responses of the in- and excurrent siphons was not measured, but it was about one-quarter to one-half second.

Just what the histological condition of the ganglion is at the time of restoration of the power of coördination it would be of interest to know. The neurogenesis, however, of the regenerating ganglion and its relation to restoration of physiological function is a problem which still awaits investigation.

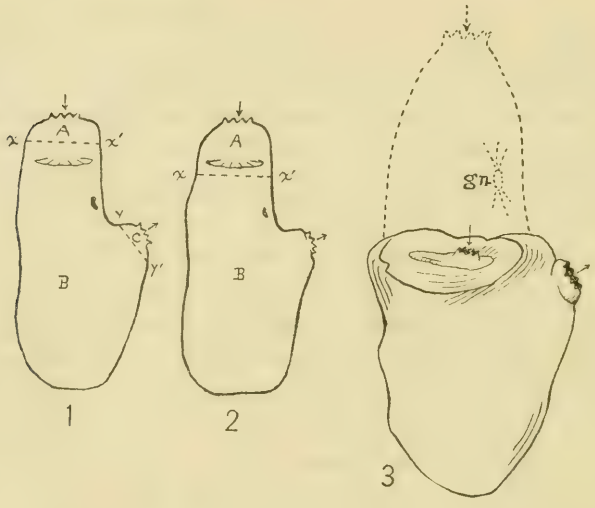
REACTION OF ASCIDIA MENTULA TO CHEMICAL SUBSTANCES

While the following are but a few incidental tests of a tentative nature, they throw a little light on the chemical sense of the animal. In the work of Hecht ('18) will be found an intensive treatment of the reactions of Ascidia atra to chemicals.

Needle-like crystals of quinine ($\frac{1}{2}$ mm. in length) were dropped on to the oral tentacles of three animals. After lying on them for a moment or two, they were ejected. A thread, knotted on

the end, weighted with a bit of tinfoil and soaked in a chemical solution of NaCl 2n, NaOH n/10, or quinine 10 per cent, and lowered on to the tentacles likewise evoked an ejection reaction.

I operated on six Ascidiae mentulae as follows: three incurrent siphons were severed proximal to the oral tentacles (fig. 2) and three distal to them (fig. 1); the excurrent siphons of the latter



Figs. 1 and 2 Two Ascidiae mentulae operated on as shown by dotted lines: in Fig. 1 the incurrent siphon (A) was amputated distal to the circlet of oral tentacles, in Fig. 2 proximal to the tentacles; excurrent siphon (6) also cut off of the first animal. Nerve ganglion is shown as a black spot near the crotch of the siphons.

Fig. 3 A regenerated Ascidia mentula. Twenty-four days after the incurrent siphon had been cut off carrying the nerve ganglion (gn) with it, a new siphon and ganglion had been regenerated and a coördinate response of both siphons was obtained upon stimulating either siphon independently.

three were also amputated a half inch from the tip. The amputated pieces opened immediately after the operation, but were unresponsive to chemical stimulation.

The following day when crystals of quinine were dropped on to the tentacles of the amputated siphons, it caused them to rise up. Crystals dropped on the lip-lobes of both incurrent and excurrent amputated siphons caused local contractions of the

lobes. In the case of the incurrent siphon, the chemical stimulation of one lobe often induced closure of the whole siphon. On one of the excurrent siphons there was also a complete closure effected. The contraction in this case was progressive in both directions from the point of stimulation around the rim of the siphon. The lip-lobes were more sensitive than the tentacles to the quinine. The inner lining of the pharynx also proved sensitive to the quinine and when stimulated brought about a contraction of the pharyngeal papillae andof ten a closure of one or both siphons.

The lip-lobes, tentacles, and pharyngeal lining were also sensitive to solutions of HCl, NaCl 2n, NaOH n/10, while to 50 per cent cane-sugar the same regions were unresponsive. The tentacles move about when stimulated, but do not contract, for they are stiff and apparently non-muscular organs. Often they gave an upward flip when stimulated. As this might have been due to the sudden ejection of water from the incurrent siphon, a siphon was amputated to include the tentacles, and the tentacles were again stimulated: they responded by rising up in a concerted reaction, but not so abruptly as before. When the ganglion was extirpated, this response of the tentacles could not be elicited. More data would probably have shown the contrary to be true for this latter case, since the erection of the tentacles occurs for an amputated siphon which is minus the ganglion and it ought also to occur for the intact siphon which is minus the ganglion.

REACTIONS OF ASCIDIA MENTULA TO VIBRATIONS

A normally sensitive individual closes its siphon to the slightest vibrations produced by disturbances in the room or by tapping on the jar in which it lies. If the incurrent siphon be amputated to include the circlet of tentacles, it is still capable of responding; but if it is excised distal to the tentacles, it no longer responds. The amputated excurrent siphon like the incurrent without its tentacles also proves to be inert to vibrations. If the ganglion be extirpated from an animal with both siphons intact, the

vibratory stimulus, if sufficiently vigorous, will still induce closure of both siphons after the effects of shock have passed.

Since both siphons respond without the ganglion and since the excurrent siphon which lacks the circlet of tentacles is about as sensitive as the incurrent siphon which has them, it would seem that neither circlet of tentacles nor ganglion is the receptive organ for the vibrations, but that the lip-lobes themselves are capable of responding to the vibrations immediately. Hecht ('18) finds the lip-lobes to be the vibration receptors in Ascidia atra.

SUMMARY OF REACTIONS OF ASCIDIA MENTULA

A. Normal animal to tactile stimulation

1. The margins of the siphons are the most sensitive part of the animal, and close when stimulated.

2. To feeble stimulation the response is local and restricted to a single lobe of the siphon. To a stronger stimulus the whole siphon closes. Further increase of strength of the stimulus produces a closure of the other siphon as well.

3. There is a short latent period of about one-half second between the responses of the two siphons.

4. The sequence of closure is reversible; the impulse travels in either direction from one siphon through the nerve ganglion to the other, depending upon which siphon is stimulated.

5. Sometimes one siphon is more sensitive than the other and takes the initiative in the response when the two are stimulated simultaneously, as by vibrations in the room.

B. Operated animals to tactile stimulation

a. With siphons amputated. 1. In a siphon partially slit longitudinally, each half responds locally to a feeble stimulus, while stronger stimulation sends the impulse around the cut and produces a response of the two halves in sequence.

2. Amputated siphons retain their sensitivity for five or six days and then die.

3. The desiphonated bodies of the operated animals recover sensitivity after the operation, live and regenerate new siphons.

4. The ganglion is not necessary to the process of regeneration of amputated siphons, as regeneration occurs even if the ganglion be amputated with the siphon. A new ganglionic mass appears in addition to the new siphon.

b. With ganglion removed. 5. Extirpation of the ganglion has two main effects: *a*) an interruption of coördination between the siphons; *b*) a reduction of tone and general irritability of the animal.

6. The ganglion regenerates in from four to six weeks.

7. Coördination is reestablished with regeneration of the ganglion, and irritability is restored to almost its original degree.

8. The new ganglion is very quickly fatigued.

C. Normal animal to chemical stimulation

Lip-lobes, oral tentacles, and pharyngeal lining are sensitive to solutions of HCl, NaCl 2n, NaOH n/10, but are insensitive to 50 per cent cane-sugar solution. Quinine crystals applied to the lip-lobes cause siphons to close; applied to the oral tentacles, they cause these to flip up.

D. Operated animal to chemical stimulation

Quinine crystals applied to the lip-lobes or oral tentacles of amputated siphons produce the same but less vigorous response as for the normal animal.

E. Normal animal to vibratory stimulation

Both siphons are sensitive to disturbing vibrations in the room.

F. Operated animals to vibrations

Amputated incurrent siphons respond only provided they are cut off to include the circlet of tentacles; amputated excurrent siphons are insensitive. Deganglionate animals respond with closure of both siphons, but a more vigorous stimulation than normal is needed to elicit the response.

SENSORY RESPONSES OF ASCIDIA ATRA LESUEUR

Since my experiments on Ascidia atra were of a preliminary character, there are only a few which may be mentioned here. For a good account of the physiology of this species reference should be made to the research of Hecht ('18). The response to various forms of stimulation are briefly as follows:

Tactile stimulation. When the outer surface of the test was stimulated with a bristle, the base and column of the animal were found to be insensitive, the necks of the siphons moderately sensitive, and the margins of the aperatures most sensitive of all. According to the strength of stimulation, the siphons respond independently or coördinately.

Vibratory stimulation. Tapping on the jar or, when the animals are in their normal habitat, stamping on the sand or clapping two stones together under water causes the siphons to close.

Chemical responses. Acetic acid 1, 0.1, and 0.01 per cent strength when pipetted on the incurrent siphon caused it to close, but had no effect on the excurrent. A weaker solution of the acid, 0.001 per cent, and distilled water were both without effect.

An animal put in one-half per cent solution of ether in sea-water became totally narcotized in eight minutes. It bent over double on itself during the process, closed its siphons, and became quite insensitive to tactile stimulation. When returned to running sea-water again, it revived in twenty to twenty-five minutes; the animal straightened somewhat, both siphons opened and responded to stimulation with a bristle.

Either siphon could be locally anesthetized by pipetting a 1 per cent solution of chloroform in sea-water on to it. With both siphons rendered insensitive in this way, the animal still exhibited the ciliary current entering the oral and issuing from the aboral siphon, and it also gave vomiting reactions from time to time, forcibly ejecting water from both siphons and thereby indicating that the body musculature was still active.

Operations. A few amputations and incisions were tried on Ascidia atra, but the animals did not live more than a day or two after the operations, due apparently to adverse laboratory conditions: owing to the presence of iron rust in the laboratory water, it was impossible to keep the tunicates alive for more than four or five days. No experiments were tried on amputated siphons. An animal, however, from which the ganglion had been excised, recovered and displayed irritability of both siphons to tactile stimulation. As the animal died shortly after, no regeneration had time to occur.

Light reactions. Both oral and aboral siphons of Ascidiaatra were tested for sensitivity to sunlight, but no evidence of a positive nature was obtained. When the animals lay in jars of running sea-water and kept in semidarkness, they closed the siphons periodically at approximately one-minute intervals, and when sunlight was focused on the siphon it could not be found to have any effect on these contraction-intervals.

EXPERIMENTS ON CIONA INTESTINALIS

For sake of comparison, a few operative experiments were performed on Ciona in which the siphons were amputated at various levels and records of reactions made for both amputated pieces and the bodies. The results are given in protocol 5 below, and by diagrams in fig. 5.

1. Effect of operative experiments on Ciona intestinalis, Animals 7, 8 and 9.

April 17, 1913. Three specimens of Ciona intestinalis were operated upon, after first narcotizing with cocain, by amputating the siphons as indicated by the dotted lines in figure 4. It will be noted that in the operation on animal 9 the nerve ganglion (black spot at the crotch) was included in the amputated piece.

From the protocol it will be noted that:

a. Ten minutes after the operation all the amputated pieces except 7 B were unresponsive to stimulation; the amputated excurrent siphon 7 B showed automatic rhythmical contractions.

PROTOCOL 5

Siphons of three specimens of Ciona were amputated as indicated in figure 4 by dotted lines

DATE	HOUR	CIONA NUMBER 7	CIONA NUMBER 8	CIONA NUMBER 9
April 17, 1913	12.20 P.M.	Siphons amputated	Siphons amputated	Siphons amputated
April 17, 1913	12.30 P.M.	Piece A, no reaction	Piece A, no reaction	Piece A, no reaction
		Piece B, irregular automatic contractions	Piece B, no reaction	Piece A, no reaction
April 17, 1913	4.45 P.M.	A, reacts to tactile stimulus	Piece A, reacts to tactile stimulus; also automatic contractions	A, reacts to tactile stimulus; also automatic contractions
				B, reacts to tactile stimulus
		N.B.—7A and 8A have long latent period. 8A reacts locally to stimulation of lip-lobe; often required repeated stimulations to elicit a response.		
April 18, 1913	12.15 P.M.	A, no reaction	A, automatic contractions	A, automatic contractions
		B, feeble response to tactile stimulus.	B, reacts to tactile stimulus	B, reacts to tactile stimulus
April 19, 1913	4.45 P.M.	A, reacts feebly to strong stimulus.	A, reacts to tactile stimulus; also automatic contractions	A, reacts to tactile stimulus
				B, also reacts
		B, reacts to strong stimulus. Cut surfaces of body also reacts	B, reacts to stimulus	

PROTOCOL 5—*Continued*

DATE	HOUR	CIONA NUMBER 7	CIONA NUMBER 8	CIONA NUMBER 9
April 23, 1913	3.30 P.M.	A, feeble; contracts only locally on one side B, feeble contractions	A, exhibits rhythmical contractions of the oral margin	A, shrunken and inert; outside peeling off, but there are new lip-lobes apparently regenerating in the center of the mass as indicated by 8 new red pigment spots B, reacts to stimulation

b. Four hours later all of the amputated pieces were responsive, and the amputated incurrent siphons 8A and 9A also exhibited rhythmical automatic contractions.

c. The latent period of response was lengthened as a result of the operation.

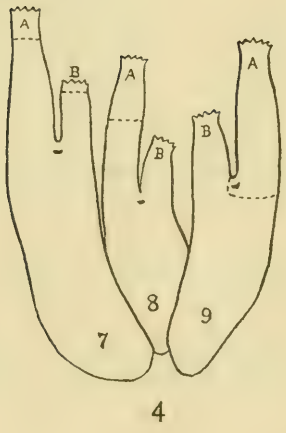
d. 9A, the piece with the ganglion included, gave visible evidence of regenerating a new siphon in the center of the degenerating amputated one.

e. B showed little progress in the regeneration of the lost parts for the same length of time.

2. Experiments with amputated siphon 8A

a. When the amputated piece was stimulated at its proximal or cut end, the impulse traveled distally and elicited a response first at the margin. This response was of two orders depending on the strength of stimulus: first, a local puckering in of one or two of the lip-lobes, and, second, a constriction of the neck region just proximal to the margin, followed by a pursing of the whole distal end. These responses are shown by diagrams 25 and 24, respectively, in figure 5. The asterisk indicates the point stimulated.

b. Rhythmical contractions occurred every minute or two involving local portions of the oral margin. These periodic contractions are shown by the series of diagrams in figure 5. The letters D and P indicate the distal and proximal ends, respectively. The arrows indicate the points at which contraction occurred.



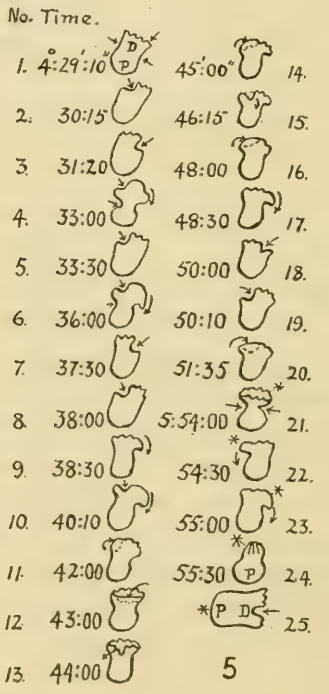


Fig. 4 Three Cionae intestinalis operated on as indicated by the dotted lines. The nerve ganglion is represented by the black spot at the crotch of the siphons. In animal No. 9 the ganglion was amputated along with the incurrent siphon.

Fig. 5 A series of diagrams showing automatic rhythmical contractions in an amputated incurrent siphon of Ciona intestinalis (8A of Fig. 4). These occurred every minute, more or less, as will be seen from time recorded in minutes and seconds to the left of each figure. The distal end of the siphon is uppermost in all figures except No. 25. D distal; P proximal. Arrows indicate the points and direction of contraction (puckering in, curling out or pursing). The asterisk means a stimulus applied at that point.

There was a certain circularity in the movement of the local contractions around the oral rim, especially seen in diagrams 15 to 20, where the invaginated part occupies successively progressive positions around the margin. These single local contractions seemed to summate in effect and to produce, after the completion of two or three, a more general reaction of the whole margin as in nos. 4, 6, 10, 21, 24.

REACTIONS OF TUNICATES TO LIGHT

From a consideration of the distribution of pigment one might well be skeptical of the photoreceptive nature of these pigmented regions. As a rule, in Ascidia mentula the pigment is restricted to a narrow interlobular band at the rim and to one or two subjacent spots, but often it is more or less extensively distributed over the siphons, sometimes coloring even the whole body surface. Another genus, Cynthia papillosa, is characteristically red all over, and Ascidia atra is entirely black; in Ciona intestinalis, again, the spots are red, while in Ascidia mammillata they are black, and in both they are localized on the margins of the siphons. From this diverse distribution and color of the pigment, therefore, one would hardly expect it to be associated with any photic sensitivity of the animal. Ascidia mentula, Ciona and Ascidia atra were tested at Naples while Ascidia atra had previously been tested at Bermuda.

The surface of the animal's body was explored with a pencil of sunlight. For the results on Ascidia atra, see page 323. The three following tables give the results for Ascidia mentula and Ciona; in tables 1 and 2 the diameter of the beam employed was 15 mm. while in table 3 it was cut down to 5 mm. The method of illuminating the animal was to reflect the beam of sunlight with a plane mirror through a cardboard diaphragm of the specified aperture upon the animal as it lay in a depth of 4 to 5 cm. of sea-water. Four regions of the body were tested in turn in this manner—incurrent siphon, ganglionic region, excurrent siphon, and the main part of the body. Contraction of the siphons was taken as indicator for the response. If no response

TABLE 1

Reaction of Ascidia mentula to a beam of sunlight 15 mm.[2] *Animals 1 and 3 were red pigmented; 2, 4, and 5 white. The ganglion of each animal was made more accessible to light by cutting away part of the tunic. The tests were made on the second day following the operation*

ANIMAL	TRIAL	INCURR. SIPHON	GANGLIONIC REGION	EXCURR. SIPHON	BODY
1	1	20″–0	20″–0	20″–0	20–0
	2	20″–0	20″–0	20″–0	
	3	20″–0	20″–0	20″–0	
	4	20″–0	20″–0	12″–+	
	5	20″–0	20″–0	10″–+	
				20″–0	
				15″–0	
				20″–+	
2	1	20″–0	20″–0	20″–0	20″–0
	2	20″–0	20″–0	2″–+	
	3	6″–+	6″–+	20″–0	
	4	20″–0	20″–0	20″–0	
	5	20″–0	20″–0	20″–0	
3	1	20″–0	20″–0	20″–0	20″–0
	2	20″–0	20″–0	20″–0	
	3	20″–0	20″–0	20″–0	
	4	10″–+	20″–0	20″–0	
	5	20″–0	20″–0	6″–+	
4	1	20″–0	20″–0	20″–0	20″–0
	2	20″–0	20″–0	20″–0	
	3	20″–0	20″–0	20″–0	
	4	15″–+	20″–0	20″–0	
	5	20″–0	20″–0	20″–0	
5	1	20″–0	20″–0	20″–0	0
	2	20″–0	20″–0	20″–0	
	3	10″–+	20″–0	20″–0	
	4	20″–0	2″–0	20″–0	
	5	20″–0	2″–0	20″–0	
Total + reactions for 25 trials		4	1	5	0

TABLE 2

Reactions of Ciona intestinalis to a beam of sunlight 15 mm.² Animal not operated on as in case of Ascidia m.

ANIMAL	TRIAL	INCURR. SIPHON	GANGLIONIC REGION	EXCURR. SIPHON	BODY
1	1	10″–0	3″–+	30″–0	15″–0
	2	30″–0	5″–+		15″–0
	3	30″–0	3″–+		15″–0
	4	30″–0	3″–+		15″–0
	5	30″–0	5″–+		15″–0
2	1	10″–0	4″–+	30″–0	15″–0
	2	30″–0	6″–+		3″–+
	3	30″–0	5″–+		15″–0
	4	30″–0	5″–+		15″–0
	5	30″–0	9″–+		15″–0
3	1	6″–+	6″–+		15″–0
	2	30″–0	5″–+		15″–0
	3	30″–0	8″–+		15″–0
	4	30″–0	6″–+		15″–0
	5	12″–+	–		15″–0
4	1	10″–0	4″–+	8″–+	15″–0
	2	30″–0	3″–+		15″–0
	3	30″–0	4″–+		15″–0
	4	6″–+	5″–+		15″–0
	5	30″–0	5″–+		15″–0
5	1	10″–0	3″–+	30″–0	15″–0
	2	30″–0	4″–+		15″–0
	3	30″–0	3″–+		15″–0
	4	30″–0	4″–+		15″–0
	5	21″–+	2″–+		15″–0
6	1	10″–0	4½″–+	5″–+	15″–0
	2	30″–0	3½″–+		15″–0
	3	15″–+	3½″–+		15″–0
	4	30″–0	–		15″–0
	5	30″–0	–		15″–0
7	1	10″–0	5½″–+	8″–+	15″–0
	2	30″–0	4″–+		15″–0
	3	30″–0	6″–+		15″–0
	4	30″–0	5″–+		15″–0
	5	30″–0	9″–+		15″–0
Total + reactions		5 in 35 trials	32 in 32 trials	3 in 6 trials	1 in 35

TABLE 3

In this table the results are given for a more critical test on Ciona which was made with a beam of sunlight 5 mm.² in area

ANIMAL	TRIALS	INCURR. SIPHON	GANGLIONIC REGION	EXCURR. SIPHON
1	1	10″–0	8″–+	10″–0
	2	10″–0	7″–+	10″–0
	3	10″–0	9″–+	10″–0
	4	10″–0	7″–+	10″–0
	5	10″–0	10″–0	10″–0
2	1	10″–0	7″–+	10″–0
	2	10″–0	4″–+	10″–0
	3	10″–0	6″–+	10″–0
	4	10″–0	5″–+	10″–0
	5	10″–0	5″–+	10″–0
3	1	10″–0	8″–+	10″–0
	2	10″–0	5″–+	10″–0
	3	10″–0	5″–+	10″–0
	4	10″–0		10″–0
	5	10″–0	4″–+	10″–0
4	1	10″–0	10″–0	10″–0
	2	10″–0	10″–0	10″–0
	3	10″–0	6″–+	10″–0
	4	10″–0	8″–+	10″–0
	5	10″–0	10″–0	10″–0
5	1	10″–0	8″–+	10″–0
	2	10″–0	10″–0	10″–0
	3	10″–0	5″–+	10″–0
	4	10″–0	8″–+	10″–0
	5	10″–0	5″–+	10″–0
6	1	10″–0	6″–+	10″–0
	2	10″–0	10″–0	10″–0
	3	10″–0	6″–+	10″–0
	4	10″–0	8″–+	10″–0
	5	10″–0	5″–+	10″–0
7	1	10″–0	4″–+	10″–0
	2	10″–0	10″–0	10″–0
	3	4″–+		10″–0
	4	4″–0	5″–+	10″–0
	5	4″–0	8″–+	10″–0
Total + reactions.....		1 in 35 trials	26 in 33 trials	0 in 35 trials

Summary of tables 2 and 3

A. TOTAL REACTIONS	INCURR. SIPHON	GANGLIONIC REGION	EXCURR. SIPHON	BODY
For 15-mm.^{2} beam.....	5 out of 35 trials.	30 out of 32 trials.	3 out of 6 trials.	1 out of 35 trials.
For 5-mm.^{2} beam.....	1 out of 35 trials.	26 out of 33 trials.	0 out of 35 trials.	
Summary for both.....	6 out of 70 trials.	58 out of 65 trials.	3 out of 41 trials.	1 out of 35 trials.
B. Average time of response:				
For 15-mm.^{2} beam.....		4.2 sec.		
For 5-mm.^{2} beam.....		8.9 sec.		

was obtained before the lapse of twenty seconds, the reaction was counted as negative and recorded as 20″–0, as in trial 1, table 1, but if a contraction occurred, as in trial 4, at the end of twelve seconds, it was recorded 12″–+, indicating a positive response. In table 3 ten seconds was the time limit allowed for the response.

It will be seen from table 1 that the body of the animal gave no response out of the five times tested, the ganglionic region but 1 out of 25, the incurrent siphon 4 out of 25 and the excurrent 5 out of 25. Since all the animals were exceedingly sensitive to stimulation by tapping on the jar, the small number of responses here obtained indicate little if any sensitivity to light.

Table 2 indicates, in contrast to the results obtained for Ascidia m., that the ganglionic area of Ciona is decidedly sensitive to light, yielding 32 responses out of 32 stimulations, while the siphons and body are relatively insensitive. The body responded but once in 35 trials, the incurrent siphon 5 in 35, and the excurrent 3 times in 6 trials. Since the excurrent siphon is much shorter than the incurrent, the 15-mm.^{2} beam could not be focused upon it without its overlapping the ganglionic region somewhat, it was therefore not tested as frequently as the other parts, but left for a more critical test with a smaller beam.

Table 3 gives the results of this test. Stimulation was made with a beam 5 mm. in cross-section. The pencil of light was small enough to afford a more restricted localization of the stimulus both on the siphons and over the ganglionic area. Under these conditions the ganglion again exhibited its decided sensitivity to light in comparison with the insensitiveness of the siphons, yielding 26 responses out of 33 trials to only one response for the incurrent and none for the excurrent out of 35 trials apiece.

From the summarized table it will be noted that when the ganglionic region was stimulated the animal gave 58 responses (closure of siphons) out of 65 trials, while to stimulations of the other regions, the incurrent siphon yielded 6 out of 70, the excurrent 3 out of 41, and the body 1 response out of 35 trails. It was the larger of the two beams of light which was effective in eliciting responses when siphons or body were stimulated. It will also be observed that the latent period of response was longer for the smaller beam (8.9 seconds) than for the larger one (4.2 seconds); i.e., the larger the area stimulated, the shorter is the reaction time.

When the body region was illuminated with a beam about 4 cm. in diameter, it squirmed and contracted in that region, while both siphons remained open. Only two of the five Cionae responded in this manner, however. The reaction time in the one case was 18 seconds and in the other 26 seconds. Although the animals lay submerged in a depth of $4\frac{1}{2}$ cm. of sea-water, this comparatively slow reaction time may have been due to the thermal effect rather than to the actinic effect. A thermometer placed at that depth and illuminated for twenty seconds showed a rise of temperature of 0.2°C., but no experiment was performed to decide the point in question.

SUMMARY OF THE RESULTS OF PREVIOUS INVESTIGATORS

Since Hecht ('18) has carefully reviewed the literature of the subject, I give below, from my own reading, only a brief summary of the results of both neurohistological and physiological studies made by earlier investigators.

A. *Neurohistological observations (Van Beneden and Julin, '84)*

1. The ganglion consists of a central fibrillar substance surrounded by a peripheral layer of cells.
2. The largest cells lie to the outside.
3. There are localized groups of cells chiefly at the anterior end and on the ventral side of the ganglion which are suggestive of motor centers.
4. Large nerve trunks run forward to the oral and backward to the aboral siphon.
5. These break up into branches to form a nerve net about the muscle fibers.
6. There are said to be club, brush, and plate-like motor nerve-endings present.
7. No specialized sensory nerve-endings have been described.
8. A cord of large ganglion cells extends down into the viscera.
9. The ganglion develops from the cerebral vesicle of the larva.
10. The visceral cord develops from the epithelial wall of the central canal of the larval nervous system.
11. The regenerating ganglion develops out of a derivative of the same embryonic tissue from which the original ganglion developed. (Schultze, '00).

B. *Physiological observations (for Ciona intestinalis)*

12. The siphons are the most sensitive parts.
13. Rapid stimulation (prodding) is more effective than slow continued pressure for the same intensity of stimulus (Polimanti, '10).

14. Duration of contraction increases with increase in the strength of stimulation (Kinoshita, '10).

15. The aboral siphon is the most sensitive, judged by the shortness of the latent period; but judged by the duration of contraction, the oral is the most sensitive (Polimanti, '10).

16. Successive stimulations cause both a decrease in vigor and duration of contractions (Kinoshita, '10).

17. Contraction of the siphon begins at the tip and travels downward, while relaxation begins at the base and travels upward (Polimanti, '10).

18. There are four types of reflexes: 1) the individual, involving only the local region stimulated; 2) the protective, in which the impulse travels swiftly from siphon to siphon via the ganglion; 3) the general, which spreads more slowly from siphon to siphon via the base of the animal; 4) the ejection reflex (Jordan, '08).

19. Narcotizing reagents are: cocain, magnesium sulphate, chloral hydrate, aceto-chloroform, quinine sulphate, nicotine, hydrochloride (Kinoshita, '10), and morphine in weak doses (Polimanti, '10).

20. Excitative reagents are: Strychnine, and morphine in strong doses (Polimanti, '10).

21. Curare is a partial narcotic according to Kinoshita ('10), but an excitant according to Polimanti ('10).

22. As to the effect of temperature: 30°C. causes frequent opening and closing of siphons; 32° begins to have a benumbing effect, and 35° causes animal to shrink and become unresponsive (Polimanti, '10).

23. For the effect of light only negative results were obtained by Kinoshita ('10).

24. Extirpation of the ganglion produces four chief effects: interruption in the coördination of the siphons, decrease of sensitivity, increase of latent period and a lengthening of the duration of contraction (Kinoshita, '10; Polimanti, '10).

25. The ganglion regulates reflexes in a feeble way either by inhibiting or facilitating them (Jordan, '08; Polimanti, '10).

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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SPECIFIC MODIFIERS OF EOSIN EYE COLOR IN DROSOPHILA MELANOGASTER[1]

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TWO DIAGRAMS

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INTRODUCTION

The main significance of the material presented in this paper is in its bearing on the question of modifying genes and the correlated question of the method by which selection attains its results. A demonstration has been made of eight mutant genes which by themselves produce little or no effect upon the eye color of flies homozygous for them, yet which modify the eye color of the sex-linked mutant 'eosin.'[2] These 'specific' modifications are clear and simple cases of multiple genes. Each is

[1] Contribution from the Zoölogical Laboratory of Columbia University, and the Carnegie Institution of Washington.

[2] A reference to the case of cream II and its bearing on the question of multiple and modifiying genes has been made in "The Mechanism of Mendelian Heredity," page 203. A brief account of the discovery and the main facts of the inheritance of cream a, dark, whiting, and cream b has been given in "Non-disjunction as proof of the chromosome theory of heredity," Genetics 1, page 147 ff. The importance in evolution of specific modifiers, typified by cream a and whiting, was emphasized in "The Critique of the Theory of Evolution," pages 163 and 164.

the result of the coaction of a specific modifying gene (cream a, b, etc.) and of a particular gene (eosin) which serves as a base or chief factor. The scale of the modifications of eosin produced by these several modifiers ranges on the one hand to a deep pink darker than eosin, and on the other hand to a pure white, without considering the effects of combinations of two or more modifiers. In origin these modifiers were entirely independent of one another, and the order of their occurrence bears only a random relationship to the dark-light seriation. By combination of several of these simple modifiers a multiple heterozygous stock could be obtained which would be amenable to selection and which would offer upon analysis a satisfactory parallel to such a case as that shown by Castle's hooded rats.

CREAM A

A pure stock of the sex-linked eye-color eosin shows a strong sexual bicolorism, that is, the eye color of the eosin female is a rather deep pink, only slightly yellowish, while the eye color of the eosin male is a pinkish yellow, much lighter in tone than the color of the female (see Morgan, '12, for an account of the origin of eosin and a colored plate showing this difference in color). Eosin females and males maintain this constant difference in color wherever they reappear after crossing, and all double recessives involving eosin, for example, eosin vermilion and eosin pink, are likewise bicolored (Morgan and Bridges, '13). Eosin is allelomorphic to white—an eosin female mated to a white male gives in F_1 no wild-type (red-eyed) daughters, but only daughters (white-eosin[3] compounds) which are intermediate in color between white and eosin. The sons of this cross are of the regular light color, which happens to be the same as that of the white-eosin female.

In carrying on the stock of non-disjunction, such a mating as that just described had been repeated through several generations when it was noticed (July 15, 1913) that the flies in one of these

[3] We denote compounds by the use of the hyphen and do not use the hyphen for non-allelomorphic multiples, for example, white-eosin, but eosin vermilion.

cultures (n43, Bridges, '16, p. 147) were somewhat variable in eye color. Certain of the flies were lighter than normal, having markedly less of the pinkish tinge than standard eosin, so that they were of a pale yellow or 'cream' color. Some of the cream males were bred to rather light white-eosin sisters which were suspected of being creams also. This mass culture gave a considerable proportion of creams, though whites (males) and eosins were also present. Little effort was made to separate the several classes of females. Among the sons there was no confusion, there being the three classes, white, eosin, and the new light color, cream.

It was now assumed that the cream was a double recessive, that is, eosin plus a recessive mutant eye color, which by analogy with the known dilution effects produced on eosin by such light eye colors as vermilion and pink, was presupposed to be a color more transparent than the normal red of wild flies.

When an eosin male is outcrossed to a wild female, all the F_2 females are wild-type, but half of the males are eosin and the other half wild-type. If a cream male is an eosin male modified by an autosomal recessive mutant as suggested above, then in F_2 from such a cream male by wild female, the new color should appear in a fourth of each of the classes which occur in the above cross. The F_2 females should be in the ratio of 6 wild-type : 2 with the new eye color, while the F_2 males should be 3 wild-type : 1 new color : 3 eosin : 1 'cream,' the double recessive.

TABLE 1

P_1, cream ♂ × wild ♀; wilde ♀ F_1 wild-type ♀ × F_1 wild-type ♂

1913, 10/7[1]	WILD-TYPE ♀	WILD-TYPE ♂	EOSIN ♂	'CREAM' ♂
I 113	104	47	44	14

[1] Date on which the culture began to hatch.

The F_2 results of the cross of a cream male to wild female (table 1) showed that while the 'cream' character reappeared in F_2 in the expected proportion (1 in 16), none of the other flies differed in eye color from those expected in the F_2 from the simple

cross of eosin by wild. That is, none of the F_2 females, all of which were not-eosin, showed a trace of dilution, and likewise all of the not-eosin males were perfectly normal in color. About one-quarter of the eosin males were modified in eye color and showed the cream color of the grandfather. It is evident that the assumption was correct that the character cream is a double recessive, the product of the action of a recessive autosomal gene added to the effect of the sex-linked gene eosin. However, this gene does not by itself produce any visible effect, as is proved by the fact that a quarter of the wild-type flies must have been homozygous for it (not eosin creams[4]) and yet all were of normal undiluted color. The case of cream a was the first in Drosophila for which such a relationship was shown, and the class of genes typified by cream a has been called specific modifiers. Such specific modifiers have since been found to be very frequent and underlay many of the difficult early cases which were amenable to selection.

The cream a gene is probably autosomal rather than sex-linked, as shown by the fewness of the creams; if the cream gene were sex-linked, then, because of the linkage between eosin and cream, from a quarter to nearly all of the eosin F_2 males should have been modified to cream, the frequency depending on whether the locus of the cream gene were far from or close to that of eosin.

[4] It is our custom to name mutants on the basis of the change they produce from the type of the wild fly, but since in the case of cream the stock homozygous for the gene is indistinguishable from the wild stock, the name was given from the most striking characteristic, namely, the power to dilute eosin to 'cream.' This mutant was not called cream a (symbol c_{ra}) until after the appearance of a second cream whose gene was found to be in the second chromosome. This new cream was then called cream II (the Roman II designating the second chromosome) and the first cream was renamed cream a (the a, b denoting simply relative order of discovery). For convenience in the further discussion of all these creams, the term 'cream' when referring to the *character* will indicate the double mutant form, eosin cream ($w^e w^e\ c_r c_r$), and in those infrequent cases in which we refer to flies homozygous for the cream gene, but not for eosin, the absence of the eosin character will be denoted by 'not-eosin' (WW $c_r c_r$ or $Ww^e\ c_r c_r$).

CREAM II

Shortly after the discovery of the first cream (cream a) a second cream appeared (September 15, 1913) among the eosin males and females of a stock culture of lethal 2. Wild-type females heterozygous for lethal 2 had been crossed to eosin miniature males, and the F_1 wild-type daughters again crossed to eosin miniature males. The mothers of the culture which gave the creams were therefore wild-type females heterozygous for eosin and miniature as well as lethal 2 $\begin{pmatrix} w^e & + & m \\ + & 1_2 & + \end{pmatrix}$ while the fathers were eosin miniature. The cream males and females which appeared were much paler than cream a, though like cream a they were a light, translucent yellow with little or no pinkish tinge.[5] None of the not-eosin flies were different in color from normal red flies.

A careful examination of the stock of eosin miniature failed to show any flies that did not have the standard eosin eye color and no lighter eye color has ever subsequently shown itself in this stock. It is evident that the gene for the modification had been present in the wild-type flies of the lethal 2 stock, but had been unsuspected so long as eosin was not present as a base. The demonstration that the cause of the observed dilution of eosin was a gene behaving in inheritance like the other mutant genes was easily made.

One of these cream males was outcrossed to a wild female. Among the F_2 flies the creams reappeared, and, as in the parallel case of cream a, the not-eosin flies were all indistinguishable from one another and from wild flies in color. The F_2 result resembled that obtained with cream a, except that, as stated, the new cream was considerably paler; and it was further discovered that besides the creams approximately 50 per cent of the eosin males were intermediates between eosin and this cream, that is, cream II diluted eosin even in heterozygous form, so that the eosin sons were visibly as well as genetically in the ratio 1 eosin : 2 eosin

[5] Colored figures of the unmodified eosin, and of cream II, of cream b, and of pinkish are being published by Bridges and Morgan in Carnegie Publication no. 287.

heterozygous for cream II : 1 eosin pure for cream II. The entire ratio, disregarding sex, approximated 12 : 1 : 2 : 1, the 12 being the red-eyed flies.

From the F_2 a few cream males were selected and bred to their sisters, all of which were wild-type in appearance, though a quarter of them were homozygous for the cream gene (not-eosin creams). This mass culture gave the expected cream females and males from which a pure breeding stock was made up. There was a difference in the color of the males and females of this pure stock, the difference being of the same order as the normal bicolorism of eosin. A complete separation of the eosin from the eosin heterozygous for cream had not been attempted in the original F_2 culture. In order to observe the heterozygous condition more closely, a cream male from the pure stock was outcrossed to an eosin female. The F_1 flies, both males and females (culture M688) intermediate males 73, intermediate females 88,[6] were lighter in eye color than standard eosin, though the difference between eosin and these heterozygotes was less than the difference between the heterozygotes and the pure cream.

Among these F_2 offspring (table 2) there were six different eye colors: among the males the same three that occurred in the original F_2 and among the females three colors which, though corresponding genetically to the classes among the males, were darker in eye color. The cream female is lighter than the normal eosin male, while the heterozygous cream female is somewhat darker than the eosin male. In order from the darkest (a deep slightly yellowish pink) to the lightest (a pale translucent yellow) the six

[6] One of the 88 intermediate daughters had only three segments to her abdomen instead of the usual five. This female (figured by Morgan, '15, p. 425, text figure 3a) was the founder of a new type of abnormal segmentation of the abdomen —'patched.' The segments were reduced in number (as in the first specimen) or more typically were cut sharply into oblique or triangular pieces which were patched together as illustrated in Morgan's figures b-f. This character was recessive, but it generally reappeared in very much less than a quarter of the F_2 offspring. The usual causes for such deficiencies are poor viability, partial or complete dependence for realization on the coaction of one or more other genes, or failure to be developed in all the flies genetically pure for the gene, whether from environmental differences or because the normal fluctuations of the character overlap the wild-type.

colors are: eosin female, heterozygous cream female, eosin male, heterozygous cream male, cream female, cream male (diagram 1, page 273). The females were first separated from the males. Then in each sex the pure creams were separated from the others, and finally the more difficult separation of heterozygous cream from eosin was undertaken. The separation of the creams from other colors is accurate, but the final separation, that of the heterozygous creams from the eosins must be regarded as only a close approximation. The sharp 1 : 2 : 1 ratio (160 : 318 : 148) which was obtained from this separation probably represents among the eosins a fair number of the darkest heterozygotes, while the lightest of the pure eosins were likewise classified among

TABLE 2

The F_2 offspring given by F_1 pairs from the cross of a cream II male to an eosin female

1913, 12/8	FEMALES			MALES		
	Eosin	Heterozygous cream	Cream	Eosin	Heterozygous cream	Cream
M77	30	57	29	29	46	25
M78	19	49	16	14	30	15
M79	23	43	19	18	34	20
M95	14	32	11	13	27	13
Total	86	181	75	74	137	73

the heterozygotes. Probably 10 per cent of the combined eosin and heterozygous cream class overlapped enough so that the separations might or might not correspond to genetic differences. One test of the correctness of the classification of intermediates was made. From culture M79 a heterozygous male and a heterozygous female were selected, and the results (culture M95) showed that both individuals were of the supposed type.

No attempt has been made to secure a stock homozygous for the cream gene but without eosin. The cultures and experiments in which such not-eosin creams must have constituted one-fourth of the wild-type flies prove that such a stock could not be distinguished by inspection from a wild stock.

That the action of cream II is specific only to eosin was suggested by crosses of cream with vermilion (x chromosome) and with pink (third chromosome). A careful examination of the F_2 flies from these crosses showed no dilution of ether vermilion or pink by the cream, that is, the double recessive vermilion cream and pink cream (not-eosin) are indistinguishable from vermilion and pink, respectively.

The proper method of study for cases of multiple factors or of modifiers is by means of linkage experiments, whereby all guesswork as to the number and effect of modifiers can be eliminated. In Drosophila such a study is rendered particularly easy by the small number of chromosomes and by the fact that in the male there is no crossing over of any of the chromosomes. In consequence, if two recessive genes which belong to the same chromosome, for example, black and vestigial of the second chromosome, enter the cross from opposite parents ('repulsion') the F_2 never shows flies which have both these mutants at the same time. The double recessive class is entirely unrepresented, and the 2 : 1 : 1 : 0 ratio of 'absolute repulsion' results. This ratio holds whatever may be the amount of crossing over in the female, for the lack of double recessive sperm prevents the double recessive eggs from revealing themselves. This ratio is in marked contrast to the 9 : 3 : 3 : 1 ratio which obtains when the two genes belong to different chromosomes, for example, curved of the second chromosome and ebony of the third chromosome.

The light color cream was known to be eosin plus a recessive modifier which belonged to an autosome linkage group. To find whether this group was that of the second chromosome, a cream male (from pure stock) was outcrossed to a curved female, curved being a recessive wing character whose gene is known to belong to the second chromosome (Bridges and Sturtevant, '14). A pair of F_1 wild-type flies inbred gave the results of table 3.

Since cream only shows itself where eosin is already present, we may disregard all the flies of culture 70 except those with eosin eyes. These eosin flies are obviously in the ratio 2 : 1 : 1 : 0 which is expected if the cream gene is in the second chromosome, though the flies a troeo few to prove the point.

TABLE 3

F_2 from the cross of cream II male to curved female

1914, 2/20	NOT-EOSIN (♂+♀)		EOSIN (MALES ONLY)			
	Wild-type	Curved	Eosin	Eosin curved	Cream II	Cream II curved
70	155	63	37	14	15	0

A more efficient experiment than this last was carried out by making all the flies of the experiment eosin, in which case the 2 : 1 : 1 : 0 ratio involved all the offspring, rather than only a quarter as in culture 70. A stock of eosin black was made up (black being a second chromosome mutant) and a female of this stock was outcrossed to a cream II male. The F_1 and F_2 results are given in table 4. All of the F_1 flies and half the F_2 flies were of the intermediate color of the heterozygous cream. In the F_2 these intermediates were classified along with the pure eosins so that the cream was treated as though a strict recessive.

The F_2 ratio of 442 : 217 : 207 : 0 is a very close approximation to a 2 : 1 : 1 : 0 ratio and proves that the gene for cream is in the second chromosome (cream II).

TABLE 4

P_1 mating, cream II ♂ × eosin black ♀ ; F_1 mating, heterozygous cream II ♀ × F_1 heterozygous cream II ♂

F1 CULTURES 1914, 3/16	HETEROZYGOUS CREAM II ♀♀				HETEROZYGOUS CREAM II ♂♂			
119	51				41			
329	15				18			
Total.....	66				59			
F_2 CULTURES 1914, 8/3	EOSIN		EOSIN BLACK		CREAM II		BLACK CREAM II	
	♀	♂	♀	♂	♀	♂	♀	♂
372	50	38	24	15	14	25	0	0
398	57	42	18	31	19	19	0	0
399	69	79	36	43	43	39	0	0
400	48	59	33	17	24	24	0	0
Totals.....	224	218	111	106	100	107	0	0
	442		217		207		0	

A similar experiment in which cream was crossed to eosin ebony (ebony being a third chromosome mutant, Sturtevant, '14) gave a typical 9 : 3 : 3 : 1 ratio (table 5), which agrees with the fact that the cream gene is not in the third chromosome.

In order to find the locus of cream within the second chromosome, it would have been necessary to run linkage experiments in which all the flies were eosin; thus, one of the experiments might have been cream II by eosin black and a backcross of the F_1 female to black cream males, and another a similar backcross in which curved was used in place of black. The amount of crossing over between black and curved was known to be about 25 per cent. The two values black cream and curved cream which would be found by two such experiments (both values might, of

TABLE 5

F_2 from the cross of cream II ♂ by eosin ebony ♀

1914, 3/31	EOSIN	CREAM II	EOSIN EBONY	CREAM II EBONY
154	61	21	18	4
161	85	28	35	14
162	134	37	36	10
Total.....	280	86	89	28

course, be found from a single more complicated experiment) would enable the locus of cream to be calculated with considerable accuracy. While much is to be learned of the mechanism of crossing over from a study of the relative distributions of loci within various regions of the chromosome, yet in the case of cream II it was thought that the compensation would not be worth the effort. Any further use of cream II in other linkage experiments would involve the 'eosinization' of all the stocks used. In the case of certain of the later creams, an approximate location of the gene within the chromosome has been made, but such location was made less laborious by the discovery of certain dominant mutations which were not available at the time the work on cream II was finished.

DARK AND WHITING

It was noticed (September 23, 1913) that in certain cultures (M 100, M 101) which were part of the experiments on non-disjunction, there were present eosin males which were abnormally dark in color. In the next generation more dark males appeared and some of the white-eosin exceptional daughters were nearly as dark as pure eosin females (no pure eosin females could occur in this culture except by the very rare equational non-disjunction; Bridges, '16). In subsequent generations the dark modification continued to reappear in such manner and in such proportions as to suggest that it was a converse case to that of cream a, that is, that there was present a recessive gene, a specific darkener of eosin, which does not modify the color of those flies which are not eosin. No very extensive records of the behavior of dark were

TABLE 6

The occurrence of whiting among the offspring of a pair of 'dark' eosin flies

1913, 11/21	EOSIN ♀	EOSIN ♂	WHITE ! ! ♀	WHITE ! ! ♂
M67.....	76	56	11	27

made, since my attention was soon diverted from this type by the occurrence in the same cultures of a much more striking variation.

One of the dark females, supposed to be a white-eosin compound, mated to a dark eosin brother gave a surprising result in that there were produced some females which were pure white in eye color (table 6). When these white females began to appear they were set down as examples of equational non-disjunction, but as the culture produced more and more of them it was seen that they were appearing in far too great a proportion to be easily explained by this rare phenomenon. Again, if the mother of M67 were indeed heterozygous for the sex-linked white, then the white sons should be equal to the eosin sons in number, instead of which they were only about half as numerous. Furthermore, half of the daughters should be pure eosin and half the lighter white-eosin compound, while in fact they seemed all to be pure eosin

with many of them of an extra dark color. This extra dark color likewise occurred in a fair proportion of the eosin sons. The whole result of culture M67 seemed inexplicable unless it was assumed that the white which here appeared was not the original sex-linked white which had been running through the experiments up to this point, but was a new and complex color.

The disappearance of the true white is easily accounted for when one remembers that the culture from which this supposed white-eosin female was drawn was extraordinarily complicated as to eye-colors by the presence of eosin and 'dark' eosin, of white-eosin compounds and of 'dark' white-eosin compounds, of the true white and of the new complex white, and as to inheritance by the occurrence in the same culture of two sex-linked and of two non-sex-linked characters, by the multiple allelomorphism of white and eosin and by the occurrence of exceptions by non-disjunction. Because of the dark modification it had been impossible to distinguish between the normal eosin color and a white-eosin compound raised to about this same color by the action of the dark modifier, and the mother chosen had really been pure eosin and not a dark white-eosin compound as supposed.

The true nature of the new white was not fully established until one of the white females from M67 was outcrossed to a bar male with the result shown in table 7. Here the surprising fact came to light that the white female when outcrossed gave no white sons, and the 'exceptional' daughters likewise were not white, but were eosin. These facts prove that the white mother did not carry the sex-linked white, but was a modification of eosin, and that the modifier was an autosomal recessive. These conclusions were confirmed in the clearest fashion in the next generation by the offspring of culture M92 whose parents were a regular bar daughter and an 'exceptional' bar son both from M74 (table 7).

The colorless flies reappeared only among those sons which were already eosin, while the not-eosin flies, both males and females, showed no effect whatever of the action of the modifying gene. The F_2 was a good example of a 12 : 3 : 1 ratio. The new gene then differs from the two creams and dark only in this

respect, that the double recessive eosin 'whiting' is pure white in eye color instead if being a pale yellow. Whiting, though much more extreme in its effects than cream II, was found to be completely recessive, the eosin males heterozygous for whiting were indistinguishable from the pure eosin males.

The results of these last two generations show conclusively that there was no sex-linked white present, and this proves that the colorless flies of the former culture M67 were likewise (eosin) whiting, the 132 eosin to 38 colorless flies of M67 being an approximation to the 3 : 1 ratio expected for a Mendelian recessive.

TABLE 7

The F_1 offspring of a white ! ! ♀ from M67 which was outcrossed to a bar male

1913, 12/10	REGULAR OFFSPRING		EXCEPTIONS BY NON-DISJUNCTION	
	Bar ♀	Eosin ♂	Eosin ♀	Bar ♂
M74	95	81	7	3

The F_2 results obtained from a bar ♀ and a bar ♂ from the above; the bar character has been ignored

1913, 12/27	Red ♀	Red ♂	Eosin ♂	White ! ! ♂
M92	217	111	64	24

The F_3 results obtained from a red female and a white! ! ♂ from the above

1914, 1/21		Wild-type	Eosin	White ! !
M114	♀	44	24	26
	♂	35	22	29
Total		79	46	55

None of the females of culture M92 were eosin and accordingly none could show whiting. In order to obtain whiting females with which to make up a pure stock of whiting, several of the wild-type daughters were bred to their whiting brothers. A quarter of these wild-type daughters must have been pure for the whiting gene, a half heterozygous, and the remaining quarter free from it. One such selected female proved to be heterozygous for both eosin and whiting, and the cross of this female to the whiting brothers was thus a backcross test (table 7).

Expectation called for an equality of the four genetic classes which occurred in culture M114, but as the not-eosin whiting males and females are indistinguishable from the wild-type flies, the 1 : 1 : 1 : 1 ratio became converted into a 2 : 1 : 1 ratio. From the whiting females and males which appeared in culture M114 a pure stock of whiting was made up. This double recessive stock was indistinguishable in appearance from the stock of the sex-linked white.

Two of the whiting males of culture M114 were tested by outcrossing to eosin females. The F_1 flies were standard eosin in color (table 8), and the F_2 raised from two pairs of these F_1 flies

TABLE 8

The F_1 and F_2 results from the outcrossing of two whiting males of culture M114 to eosin females of pure stock

1914, 1/27	EOSIN ♀		EOSIN ♂	
M118.....	64		66	
M118.1.....	38		35	
Total.....	102		101	
1914, 2/9	**EOSIN ♀**	**EOSIN ♂**	**WHITING ♀**	**WHITING ♂**
25	118	120	50	61
26	118	104	53	53
Total.....	236	224	103	114

was of the ordinary 3 : 1 type, the whiting reappearing among the females and males equally (table 8). It is evident that whiting has excellent viability, for in most of these cultures it slightly exceeds expectation, the greatest departure being in the above cases where it reappeared as 32 instead of 25 per cent of the F_2 flies.

The sex-linked genes white, eosin, and cherry are all allelomorphs of each other. Cherry and eosin females are almost identical in color, but cherry males are of the same color as cherry females and are easily separated from eosin males. A series of tests was run to observe the effect of the whiting gene when acting with these allelomorphs. The previous experiments in which

homozygous whiting had no visible effect in the absence of eosin have proved that red, the normal allelomorph of white, eosin and cherry, is unaffected.

In order to observe the interaction of white and whiting, two (eosin) whiting males from M114 were out-crossed to white females of pure stock. The F_1 females were white-eosin compounds of normal color, and the sons were white as expected (table 9). F_2 from the cross of eosin male by white female gives in equal numbers the four classes: white-eosin ♀, white ♀, eosin ♂, and white ♂. The cross of (eosin) whiting

TABLE 9

The F_1 and F_2 offspring from the cross of (eosin) whiting males to white females

1914, 1/20	WHITE-EOSIN ♀	WHITE ♂
M101	113	107
M117	46	46
Total	159	153

1914, 2/3	WHITE-EOSIN ♀	(WHITE-EOSIN) WHITING ♀	WHITE ♀	WHITE (WHITING) ♀	EOSIN ♂	(EOSIN) WHITING ♂	WHITE ♂	WHITE (WHITING) ♂
9	44	81			54	75		
31	37	65			24	55		
32	18	21			25	28		
Total...	99	167			103	158		

male by white female should give this same result with the further genetic subdivision of each of the four classes into 3 not-whiting to 1 homozygous for whiting. Since all white flies are incapable of further dilution the white whiting double recessive should be white even in the absence of effect of whiting on white. But in the white-eosin compound females we shall have a chance to observe the interaction of white and whiting. If white and eosin react in the same fashion toward the whiting gene, then the substitution of one white allelomorph for one eosin in the white-eosin compound should not prevent the whiting from diluting the compound to a colorless eye. But if the white cannot thus be substituted, the white-eosin compound should

be either unaffected or less affected by the action of the whiting gene. Should the white-eosin whiting flies be colorless, then the colorless classes should be 3 white + 1 whiting white + 1 eosin whiting (eosin whiting if males, white-eosin whiting if females), and these classes should total $\frac{5}{8}$, or 62.5 per cent. The colorless flies did in fact constitute 61.7 per cent. What is even more significant, the proportion of colorless flies among the females was just as great as among the males (table 9). The colorless males are known to include the (eosin) whiting flies, and the conclusion is justified that the like class of colorless females included white-eosin whiting flies.

TABLE 10

The F_1 and F_2 offspring from the outcross of (eosin) whiting males to cherry female

1914, 1/28	EOSIN-CHERRY ♀	CHERRY ♂
M119	64	75
M120	64	63
Total	128	138

1914, 2/9	EOSIN-CHERRY ♀	EOSIN-CHERRY (WHITING) ♀	CHERRY ♀	CHERRY (WHITING) ♀	EOSIN ♂	(EOSIN) WHITING ♂	CHERRY ♂	CHERRY (WHITING) ♂
27			159		64	12	93	
28			169		48	24	73	
Total...			328		112	36	166	

The most interesting of these three experiments was that in which (eosin) whiting males were outcrossed to cherry females. The F_1 females were eosin-cherry compounds, and the males were cherry which is of nearly the same color (table 10). F_2 of the cross of eosin male by cherry female gives in equal numbers the F_2 classes eosin-cherry compound ♀, cherry ♀, cherry ♂, and eosin ♂. The eosin-cherry female and the pure cherry female are so nearly the same color that they form one phenotype, but the eosin males can be easily separated from the cherry males. The cross of (eosin) whiting male by cherry female will give further subdivisions of each of these classes into 3 not-whiting: 1 homozygous for whiting.

The result obtained in this F_2 was entirely unexpected; for cherry, which is allelomorphic to eosin and so closely similar as to be distinguishable only in the males, gave a totally dissimilar reaction with whiting, the double recessive, cherry whiting, being indistinguishable from cherry both in males and females (table 10). This result is very clearly shown by the males of this F_2 which closely approach the ratio 4:3:1. A corresponding condition is revealed in the females, for not a single colorless female occurred, and though the females were of four main genetic types they were objectively of but a single class. The whole ratio in this F_2 was then 12:3:1 exactly as would be the case in the F_2 from an outcross of (eosin) whiting male by wild female; that is, cherry is similar to the wild-type allelomorph red in its interaction with the whiting gene. It was observed in the F_2 from (eosin) whiting by white that the eosin-white whiting female is colorless; in this experiment we observe that the eosin-cherry whiting female is not thus diluted. This comparison shows that there is here a total dissimilarity in the reaction of the allelomorphs white and cherry. The fact that cherry whiting is indistinguishable from cherry proves a like dissimilarity of the allelomorphs eosin and cherry. The entire group of experiments reveal how curiously specific whiting is in its power of dilution, giving with eosin and with white the extreme amount of dilution, and with red and with cherry the other extreme of dilution—no dilution whatever.

One further test of the lack of reaction of whiting with cherry was made without the presence of eosin in the cross. The F_1 cherry males from the cross of cherry female by (eosin) whiting sp. male were heterozygous for the whiting gene. One of these males was outcrossed to pure cherry females and from the F_1 cherry males and females two mass cultures were made (table 11). One-sixteenth of the flies hatching from these new cultures should be whiting as well as cherry, while the eosin has been entirely eliminated. None of the 678 cherry flies from these cultures was diluted, a result which confirms the conclusion that the double recessive cherry whiting is not visibly different from cherry.

TABLE 11

The F_2 offspring of two F_1 mass cultures from the outcross of an F_1 cherry male heterozygous for whiting (ex M119) to cherry female

1914, 3/2	CHERRY AND CHERRY (WHITING) ♀ ♀	CHERRY AND CHERRY (WHITING) ♂ ♂
75	215	241
95	118	104
Total.....	333	345

CREAM III

In a culture which was part of an experiment on lethal 1b (Morgan and Bridges, '16) Miss E. M. Wallace found (February 27, 1914) a few females which were much lighter in eye color than their white-eosin sisters. Following my suggestion that this color might be a cream similar to cream a or cream II, these females were given me for experimentation. I outcrossed one of them to an eosin male of pure stock. The F_1 males (table 12) showed that this female had carried yellow and white in one X and eosin in the other, this being the condition expected from her parentage. The cream was seen to be recessive, since all of the F_1 flies were standard in color, and not sex-linked, since it did not appear in the sons of the cream mother.

An F_1 mating was made between an eosin female and an eosin male, since by this means both yellow and white could be eliminated from the experiment. In F_2 the cream color reappeared but in numbers rather fewer than the expected quarter (table 12). In appearance this cream III was very nearly as light as the homozygous cream II.

TABLE 12

The F_1 and F_2 offspring from the outcross of a new cream (cream III) ♀ to an eosin ♂

1914, 3/9	WHITE-EOSIN ♀	EOSIN ♀	YELLOW WHITE ♂	EOSIN ♂	YELLOW EOSIN ♂	WHITE ♂
92	25	21	15	24	0	0

1914, 3/26	EOSIN ♀	EOSIN ♂	CREAM III ♀	CREAM III ♂
136	91	92	22	21

From the cream males and females of culture 136 a pure stock of the double recessive eosin cream was made up. This double recessive will usually be referred to simply as cream, and 'not-eosin' will be used in other cases.

In order to find out in which chromosome the gene of this cream lies, a second cream female (found February 28 in the same culture as the first) was mated to an eosin black male of the stock which had been made up to test cream II. Two F_1 pairs (eosin ♀ x eosin ♂) gave a 9: 3: 3: 1 ratio (table 13), which proves that the gene for this cream is not in the second chromosome.

TABLE 13

The F_1 and F_2 offspring from the outcross of a cream III female to an eosin black male

1914, 3/18	WHITE EOSIN ♀	EOSIN ♀	YELLOW WHITE ♂	EOSIN ♂	YELLOW EOSIN ♂	WHITE ♂
121	52	61	35	58	0	3

1914, 4/4	EOSIN	EOSIN BLACK	CREAM III	BLACK CREAM III
159	118	45	56	15
160	136	49	41	20
	254	94	97	35

The fact that this cream is not in chromosome II makes it probable that it is in chromosome III, since chromosome IV is very small. To test this point a cream female from the pure stock made from culture 136 was mated to an eosin ebony male from the stock which had been used in testing cream II. Three pairs of eosin F_1 flies gave the approximation to a 2: 1: 1: 0 ratio observed in table 14.

The absence of the double recessive cream ebony proved that this cream was in chromosome III, and it was accordingly called cream III; the Roman III being the symbol for the third chromosome.

It was decided to find approximately the locus of cream III in the third chromosome. To do this it was necessary to find

the amount of crossing over between cream III and some two other genes whose loci were already known. The best method of finding the amount of crossing over is by the backcross, which consists in testing a multiple heterozygote by the corresponding multiple recessive. The double recessive cream ebony III was made up by breeding the eosin ebony and the cream III flies of F_2 (culture 361) to each other. Nearly all of these matings gave only eosin flies in F_1, but one pair gave half of the flies eosin and the other half eosin ebony. These eosin ebony flies were heterozygous for the cream gene since their father was homozygous cream. When these F_3 eosin ebony flies were bred together a quarter of their offspring (F_4) were cream III ebony. One of

TABLE 14

The F_2 offspring from the outcross of a cream III female to an eosin ebony male

1914, 8/3	EOSIN		EOSIN EBONY		CREAM III		CREAM III EBONY	
	♀	♂	♀	♂	♀	♂	♀	♂
361	43	42	24	23	27	18	0	0
363	56	50	19	23	16	23	0	0
371	59	57	19	22	42	26	0	0
Total.....	158	149	62	68	86	67	0	0
	307		130		153		0	

the resulting cream III ebony males was outcrossed to an eosin female and their F_1 eosin daughters were backcrossed to fresh cream III ebony males from the general stock which had meanwhile been made up and carried on (table 15).

As soon as this experiment began to produce results, a great variation in the amount of crossing over in the various cultures was noticed. The ebony stock used throughout this experiment was supposed from previous tests (Sturtevant, '13) to be homozygous for a modification of the amount of crossing over in the third chromosome. This modifier (C_{III}) when in the heterozygous form, greatly reduces the amount of crossing over, and it was this kind of result that was expected. Cultures 580 and 582 of table 15 gave a low percentage of crossing over, and in the

light of subsequent experiments these are known to be the cultures in which the mother was heterozygous for the crossover variation (C_{III} c_{III}). The other three cultures (578, 579, 581), however, gave a much higher percentage of crossing over, and for this one of two explanations seemed possible. Either the cream III ebony stock was not pure for C_{III} as supposed, in which case this high value would be comparable with the regular values obtained in experiments free from C_{III}; or the eosin stock used as P_1 (in the mating of cream III ebony male by eosin female) itself carried C_{III} (in heterozygous form, since she gave a ratio of 1:1), in which case the high crossover value is comparable with the crossover values obtained from mothers homozygous for C. While crossing over in females heterozygous for C_{III} is very much lower than in females free from C_{III}, yet in females homozygous for C_{III} it is the highest of all (Muller, '16). I was not able to decide without testing whether the high values were due to homozygous C_{III} on the one hand or to homozygous not-C_{III} on the other.

TABLE 15

The coupling backcross offspring from the P_1 mating of an cream III ebony male to an eosin female. The F_1 eosin females back-crossed singly to cream III ebony males

1914, 10/6	CREAM III EBONY	(EOSIN)	CREAM III	(EOSIN) EBONY	PER CENT CROSSING OVER
578	74	72	64	78	49.3
579	55	58	28	24	31.5
580	107	113	21	15	14.1
581	73	55	34	45	38.1
582	87	73	11	9	11.1

This question was not tested until December, 1915. Meanwhile the number of stocks of primary mutations had increased to such an extent that it had become necessary to eliminate as many as possible of those stocks which were decreed less generally useful. Among those discarded (or lost) at various times were cream a, cream II, whiting, and cream III. However, the stock of cream III ebony had been spared for this special test.

In order to see if the experiment would still give the two kinds of results observed before, several P_1 matings (cream III ebony ♂ × eosin ♀) were made, and each father was saved and remated to one of his daughters. Of six such tests five gave the low value and one the high (table 16). The low values corresponded to the expected result that the F_1 females should be heterozygous for C_{III}.

TABLE 16

The B. C. offspring of cream III ebony males outcrossed to eosin females (pairs) and then remated each to a single daughter

1916, 1/8	CREAM III EBONY	EOSIN	CREAM III	EOSIN EBONY	TOTAL	CROSSOVER
						per cent
2773 ♀	58	52	8	7	125	10.6
2773 ♂	44	64	9	2	119	
2774 ♀	87	87	19	9	202	14.6
2774 ♂	45	36	8	8	97[1]	
2775 ♀	47	55	6	7	115	13.7
2775 ♂	36	26	9	4	75	
2776 ♀	56	71	23	8	158	18.0
2776 ♂	31	33	8	3	75[1]	
2777 ♀	33	34	22	24	113	41.0
2777 ♂	35	33	24	24	116	
2778 ♀	68	76	16	6	166	15.3
2778 ♂	52	63	10	15	140[2]	

[1] The two cultures 2774 and 2776 produced only half as many sons as daughters owning to the occurrence of a lethal (lethal 11), the mutation for which must have taken place in the eosin stock some time before.

[2] Culture 2778 gave rise to a mutant 'roof wings' which likewise had originated in the eosin stock.

That backcrosses made up from the low crossover cultures continued to give only low values is shown by table 17. The occurrence of a high value among such cultures would have been an indication that one of the cream III ebony males previously used had failed to be homozygous for C_{III}.

If the high value of culture 2777 were not due to a homozygous not-C_{III} condition, then it should be due to the converse cause—homozygosity for C_{III}; that is, the eosin stock which had furnished the other parent for our original experiment had itself been carrying the linkage variation C_{III}. In this case every cream III ebony fly from the high-value culture should be homozygous for C_{III}, and should give, when outcrossed to eosin flies free from C_{III}, all offspring heterozygous for C_{III} and all daughters

TABLE 17

The B. C. (F_3) offspring given by continued breeding from a low crossover value line (2773) of table 16

1916, 2/1	CREAM III EBONY	EOSIN	CREAM III	EOSIN EBONY	TOTAL	CROSSOVER VALUES
3124 ♀	60	50	5	8	266	10.1
3124 ♂	67	62	3	11		
3402 ♀	43	61	10	7	221	16.7
3402 ♂	28	52	10	10		
3403 ♀	32	55	12	7	223	15.2
3403 ♂	41	61	10	5		
3404 ♀	18	23	3	3	108	15.7
3404 ♂	20	30	6	5		
3742 ♀	30	65	16	6	225	13.8
3742 ♂	39	50	12	7		
3743 ♀	23	39	7	2	129	14.0
3743 ♂	16	33	5	4		
Total	417	581	99	75	1172	14.8

giving the low value. One such test was made and the daughters all showed the low value (table 18).

Both of these tests (tables 17 and 18) pointed to the presence of C_{III} in the particular eosin stock used to outcross to, though they were not conclusive because of being done on so small a scale. Conclusive proof (in press, Carnegie Publication no. 287) was soon furnished by Sturtevant, who had been trying for some time to get, by crossing over a stock, which should be C_{III} but not

ebony. He made tests of the eosin stock, and found some individuals that carried C_{III}, from which he secured the desired stock.

In making the tests of the last experiment (table 18) the outcrosses of cream III were made to eosin dichaete in order to establish by a three-point linkage experiment the location of cream III in the third chromosome. The preceding experiments (tables 15, 16, and 17) had already given 404 crossovers in a total of 2887 flies, or 14 per cent, in cases where the mothers were heterozygous for C_{III}. In other experiments (by Sturtevant and Muller) in which ebony has been run with various third chromo-

TABLE 18

The B. C. offspring from the outcross of a cream III ebony female from 2777 to an eosin dichaete male, and the F_1 eosin dichaete females backcrossed singly to cream III ebony males

1916, 3/23	^{c}rIII D′ e		^{e}rIII	′D e	^{c}rIII	D′ e	^{c}rIII	D′	e	CROSS-OVER VALUE OF CREAM III EBONY
	Cream III ebony	Eosin dichaete	Cream III dichaete	Eosin ebony	Cream III	Eosin dichaete ebony	Cream III dichaete ebony	Eosin		
3902	136	146	7	6	9	10	0	1		10.2
3903	93	82	2	9	2	3	0	1		8.3
3904	86	96	1	3	10	4	0	0		9.0
Total.....	315	324	10	18	21	17	0	2		9.3

some genes in heterozygous C_{III} condition, the amount of crossing over of sepia with ebony has been 25 ±, of pink with ebony 2 and of ebony and rough 0.0+. That is, there is some crossing over in the region to the left of ebony, nearly all of which is in the sepia pink region, but almost none to the right of ebony (ebony rough 0.0+). It seemed probable from the crossover value 14.0 that cream III lies to the left (i.e., near the sepia end) and at a position between sepia and pink. A comparison of the high value for cream III ebony (41.0) with the known homozygous C_{III} values (Muller) for sepia ebony (48+) and for pink ebony (46+) led to this same conclusion though less definitely. The mutant dichaete was well adapted for this test because of

its dominance, its known position in the suspected region, and from its easy classification in the same flies with cream III, it being a wing and bristle character. The double crossover result (table 19) showed that cream III is situated to the left of dichaete. The cream III dichaete crossover value (4.2) is considerably smaller than the sepia dichaete crossover value (14.8) found in heterozygous C_{III} experiments, so that cream III is known to be between sepia and dichaete. The normal amount of crossing over between sepia and dichaete is 11.0 so that the probable position of cream III is 3.1 to the left of dichaete or 7.9 to the right of sepia which is the zero position.

TABLE 19

The B. C. offspring from the outcross of a cream III ebony female to a dichaete male, and the F_1 dichaete females backcrossed singly to cream III ebony males

1916, 8/22	RED								EOSIN							
	c_{rIII} e	D′	c_{rIII} D′	e	c_{rIII}	D′ e	c_{rIII} D′ e		c_{rIII} e	D′	c_{rIII} D′	e	c_{rIII}	D′ e	c_{rIII} D′ e	
4990	24	23	1	7	0	0	0	0	9	13	1	1	1	2	0	0
4991	35	38	1	8	3	2	0	0	33	32	0	0	1	2	1	0
4996	5	4	0	1	0	3	0	0	20	10	0	0	1	3	0	0
4997	9	13	1	2	3	0	0	0	11	17	0	0	0	0	0	0
4998	50	47	0	8	1	2	0	0	45	58	2	3	0	2	0	0
Total.	123	125	3	26	7	7	0	0	118	130	3	4	3	9	1	0

The occurrence of the linkage variation and its testing had diverted me from making tests of the effect of the cream III gene in the absence of eosin as a base. Accordingly, the preceding experiment was repeated, but in such a fashion that half of the flies should be not-eosin. A cream III ebony female from the high value stock was outcrossed to a simple dichaete male. Several of the dichaete daughters ($\frac{c_{rIII} + e\ C_{III}}{+\ D' + +}$) were back crossed singly to cream III ebony males of the high stock. The result (table 19, left side) showed that the gene responsible for the dilution of eosin to the cream color gave by itself a color somewhat similar to purple, that is, a 'magenta.' But when

separations were attempted it was found that this color was not sufficiently distinct from the red to make classification accurate. In the eosin half of the flies on the other hand, the separation was easy and entirely accurate. That is, while the so-called 'cream III' gene by itself gave a certain effect, it gave so much more marked an effect in the presence of eosin that it was decided to retain the name 'cream III' instead of renaming the mutant 'magenta.' The separations in the not-eosin half of table 19 correspond roughly to those of the eosin half, but the large size of the ebony not-magenta class is due to the impossibility of distinguishing the 'magenta' character, even though it was undoubtedly present in most of these twenty-six flies. If this character were accurately classifiable (as magenta) without first laboriously combining eosin with all the flies used in the matings of each experiment, it would be incomparably more useful even if slightly less interesting from our present viewpoint. However, an experiment planned through eight generations had to be abandoned because it was found impractical to distinguish between the cream III and the not-cream III where eosin was not present as a sensitizer. But this aborted experiment revealed that the ebony was a disturbing factor—that in the not-ebony flies the distinction was sharper than in ebony flies. The new possibility arose that the mutation could be used (as magenta) by excluding ebony from all the experiments. While this has not been adequately tested, it seems hopeful that with experience one may be able to use this eye-color without a preliminary eosinization of all the stocks, though it is not to be denied that in the presence of eosin the ease and speed of classification would be greater.

CREAM B

An eosin female from a stock of non-disjunction when mated to a bar male gave (culture 82, March 10, 1914) among the eosin sons one whose eye color was as light as that of cream II or cream III. This male was outcrossed to a wild female and in F_2 gave creams among the eosin sons, but no disturbance of the color of the not-eosin flies (cultures 183, 184, 185). The

F_2 ratio was again 12:3:1, as in similar crosses with other recessive specific dilutors. But the creams (cream b) which occurred in this F_2 were not as pale as any of the preceding creams.

From the circumstances of the appearance of cream b—that it was observed in the F_1 of an outcross and as a single individual —we should expect it to be a dominant, but as a matter of fact it proved to be a recessive. It seems probable, in explanation, that more creams were actually present in this F_1 but were overlooked, since attention was distracted by the simultaneous appearance in the same culture of still another mutation (lethal 4), and more especially since the effect of cream b is rather slight. Only occasionally was one of the F_2 creams as marked as the grandfather, and the mutation might not have been recognized at all were it not that an extreme fluctuant had attracted attention. Since cream b is recessive, we must suppose that the gene was present in both parent stocks. It could have been present in the bar stock and been undetected because of the lack of eosin, without which it has no visible effect. And the character might readily have been present in the eosin non-disjunction stock and have been passed over as an age variation, since as we ordinarily see flies from a stock culture they are of all ages and of corresponding densities of pigmentation.

A pure breeding stock of cream b was made up for use in back crossing. By this time we were in possession of a good second chromosome dominant 'star' and likewise of a perfect third chromosome dominant 'dichaete,' which mutants have now become the most important in their respective chromosomes. By aid of these two dominants it is very easy to determine in a single experiment whether a given mutant is in the second or third chromosome. Thus, in the case of cream b, a stock of eosin star dichaete was made up and used in making a P_1 cross to the cream. Then F_1 eosin males which showed both star and dichaete and which were heterozygous for the recessive cream were backcrossed to cream b females of stock. There is no crossing over in the male of Drosophila, so that if cream b were in the second chromosome, none of the B.C. (back cross) stars should be cream, while half of the dichaete should be cream and half

TABLE 20

The B. C. offspring from the P_1 mating of an eosin star dichaete male to a cream b female and the backcrossing of the F_1 eosin star dichaete male to cream b females

1916, 9/8		NON-CROSSOVERS				CROSSOVERS (IN MALE)			
		Eosin star	Eosin star dichaete	Cream b	Cream b dichaete	Star cream b	Star cream b dichaete	Eosin	Eosin dichaete
5155	♀	20	25	26	12	0	0	0	0
	♂	19	21	21	26	0	0	0	0
5409	♀	14	21	18	26	0	0	0	0
	♂	14	15	17	24	0	0	0	0
Total		67	82	82	77	0	0	0	0

not. If, on the other hand, the cream were in the third chromosome, then none of the B.C. dichaetes should be cream, while the star and cream should assort at random. The experiment proved that the gene for cream b is in the second chromosome (table 20).

An (eosin) star female and a cream b male selected from the B.C. offspring gave in the next generation the amount of crossing over between star and cream b (table 21). This value of 22.1 includes some double crossing over, and the corrected or 'map' distance is probably about 22.5. The chances are in favor of the cream b locus being to the right of star, since star happens to occupy the leftmost of the known loci.

TABLE 21

The B. C. offspring given in F_3 by an eosin star female and a cream b male from table 20

1914, 10/20		NON-CROSSOVERS		CROSSOVERS (IN FEMALE)	
		Eosin star	Cream b	Star cream b	Eosin
5593	♀	36	41	8	8
	♂	22	41	12	12
5824	♀	47	39	13	7
	♂	28	49	11	15
Total		133	170	44	42

PINKISH

In the fall of 1913 a stock of eosin black had been made up with which to test the chromosome group of cream II. In the following summer (July 27, 1914) I noticed that a few of the males were somewhat lighter in eye color than the others, but seemed chiefly noticeable because of the weakness of the yellow component of the eosin eye color. The color of the regular eosin male is a pinkish yellow; the color of creams a, II, III, and b is nearly a pure yellow with little of the pinkish tinge, while this new color was somewhat the converse of this and was pale pink with relatively little yellow.

One of these males mated to a sister gave all of the sons of this pinkish color and all the daughters of a similar color, which is, however, much harder to distinguish from standard eosin. It seems that this character is somewhat sex-limited in the same direction as eosin. Pure stock of the mutation had been obtained at once through the happy selection of a pure pinkish female which had been taken to be simply an eosin female of somewhat lighter eye color because of being freshly hatched.

Since pinkish appeared in a stock of eosin black, material was on hand to test the chromosome group at once. Accordingly, black pinkish females were outcrossed to eosin males and the F_1 eosin females, standard eosin in color, were backcrossed to black pinkish males. In the B.C. cultures half of the flies were not-black, and the not-black pinkish flies were seen to be less markedly 'pinkish' in tone than the blacks. In the absence of black the eye color was more nearly like that of the other creams, though the amount of dilution is less than in the case of any of the other creams. In the first of these B.C. cultures (table 22) males and females were both classified together. Some question having been raised as to the accuracy of the separation of pinkish from eosin among females, the cross was repeated, and the more readily classifiable males (last three cultures) gave the same result as before. It was seen that the new or crossover combinations were as numerous (51.4 per cent) as the original classes, and this independent inheritance was taken to mean that the

gene for pinkish is not in the second chromosome. While this was a mistaken notion—the true relation being that the gene is so far away from black that in the female there is entirely free crossing over—yet it led to the device of the efficient 'double mating' method of ridding a given stock of an undesired recessive.

If pinkish were in the third chromosome, then the presence of the black in the pinkish stock could be of no advantage, and might be a very serious handicap, since it would prevent the use of all our third chromosome stocks containing ebony or sooty. The first step in the elimination of black was to mate together some of the not-black pinkish flies of table 22. One-third of the not-black offspring of such pairs should be of the desired

TABLE 22

The offspring given by the F_1 eosin-eyed daughters from the outcross of black pinkish females to eosin males, when back-crossed to black pinkish males

1914, 9/23	NON-CROSSOVERS		CROSSOVERS	
	Black pinkish	(Eosin)	(Eosin) black	Pinkish
525	70	81	71	84
526	36	29	32	22
2424	25	21	24	35
2425	24	27	29	31
2426	28	24	14	29
Total.....	183	182	170	201

kind, that is, entirely free from black. Our task was then to pick out from the mixture of pure grays and grays heterozygous for black some pure gray males for outcrossing to eosin females. In this special case we were aided by the fact that black happens to be slightly dominant, that is, the heterozygous blacks are somewhat darker than the pure grays. While this difference is not marked enough to be used regularly in classification, yet it enables us to pick out by inspection a greater proportion of pure grays than we would get by random selection. Four such males were selected as being probably free from black and were mated to eosin females. Into the same bottle with each pair of these flies was put a virgin (red-eyed) black female. The offspring from these two mothers are easily distinguished, since

they are eosin-eyed if from the eosin mother and red-eyed if from the black mother. The offspring from the black mother constitute a test of whether the father were free from black, for in this case none of the red-eyed offspring hatching in the double mating culture should be black, while if the father were heterozygous for black half of the red-eyed offspring should be black. Only one of the four cultures gave black offspring, and this culture was then discarded. The eosin-eyed flies of the other three cultures were all heterozygous for pinkish and at the same time free from black. By mating together some of these eosin-eyed flies pure pinkish offspring should be obtained as a quarter of the offspring. A more efficient method, and the one actually followed, was to save the fathers and mate them to their eosin-eyed daughters, since in this case half, rather than a quarter, of the progeny should be pure pinkish.

In order to show by an actual test that the gene for pinkish is in the third chromosome, it was decided to take advantage of the fact of no crossing over in the male and to run a back-cross test of a male heterozygous for pinkish and for the dominant third-chromosome character dichaete. It was now realized that the back-cross tests of females heterozygous for pinkish and black had not excluded the possibility of pinkish being in the second chromosome, though they had shown that, if so, it could be only in one or the other end-region and not near black. Accordingly, exactly the same procedure was followed as in the tests for the location of cream b, that is, a pinkish female was outcrossed to a male which had the dominant second-chromosome star as well as dichaete. The F_1 eosin star dichaete males were then back-crossed to pinkish females. The result showed (table 23) that the gene for pinkish is in the second and not the third chromosome; for, as well as could be judged, none of the star flies were pinkish, while all the not-stars seemed to be pinkish, and dichaete was present in half of both the star and the pinkish classes.

In the light of this test, and from the fact that there was about 50 per cent of crossing over between black and pinkish, we could place pinkish in either the extreme left or the extreme right end-

TABLE 23

The B. C. offspring given by F_1 eosin star dichaete sons, from the outcross of a pinkish female to a star dichaete male, when back-crossed to pinkish females

1916, 8/25		NON-CROSSOVERS				CROSSOVERS (IN MALE)			
		(Eosin) star	(Eosin) star dichaete	Pinkish	Pinkish dichaete	Star pinkish	Star pinkish dichaete	(Eosin)	(Eosin) dichaete
5029	♀	10	12	10	9	0	0	0	0
	♂	17	13	16	22	0	0	0	0
5266	♀	22	20	18	21	0	0	0	0
	♂	20	20	21	26	0	0	0	0
Total		69	65	65	78	0	0	0	0

region of the second chromosome. Fortunately, one advantage of the test just described is that it left us in possession of females heterozygous for star and for pinkish, and a back-cross test showed (table 24) that there is very free crossing over between star and pinkish. Pinkish is known, therefore, to be in the right-hand end of the second chromosome in the neighborhood of arc, speck, balloon, etc. Had the test given almost no crossing over between star and pinkish, we should have known that the gene for pinkish was in the left end, but this was not the case.

A test as to whether the pinkish gene would have any visible effect in the absence of eosin showed (table 25) that in a very small per cent of the flies homozygous for pinkish there is a very slight dilution. This dilution is, however, so slight that rarely could one be sure that the effect observed is due to dilution rather than to the slight normal fluctuation of the red.

TABLE 24

The B. C. offspring given by a star female from table 23 when back-crossed to a pinkish male

1916, 9/23		NON-CROSSOVERS		CROSSOVERS	
		(Eosin) star	Pinkish	Star pinkish	(Eosin)
5267	♀	19	30	19	20
	♂	26	26	19	16
Total		45	56	38	36

TABLE 25

The F_2 offspring given by the F_1 wild-type females and F_1 eosin males, from the outcross of pinkish females to wild males

1916, 10/28	WILD-TYPE	(NOT-EOSIN) PINKISH)	EOSIN	PINKISH
5678	121	5	76	24
5680	52	2	46	17
5703	63	14	59	18
5704	57	6	52	26
5705	80	3	67	18
Total.....	373	30	300	103

CREAM C

While looking over the eosin stock (July 13, 1916) in search of a virgin female, I noticed that a few of the flies seemed to be unusually light in eye color. If this paleness were of genetic origin, then the gene must be of recent mutation, for on two occasions subsequent to the discovery of creams whose origin might be traced back to the eosin stock, this stock had been bred in such a way as to make it extremely unlikely that any cream or other eye-color mutation then present could fail to be eliminated.

A pale eye color due to a mutation within the eosin stock might be an allelomorph of eosin, a specific modifier of eosin, or a non-specific modifier, such as vermilion or pink. The gene for the modifier might be sex-linked or be in any of the other three chromosomes. Only one experiment was carried out with this pale eye color, but this experiment was so planned that it answered all the above points. One of the pale females was mated to a star dichaete male. The F_1 offspring showed at once that the pale color was due neither to an allelomorph of eosin nor to a sex-linked modifier, for the sons were all standard eosin in eye color. Likewise the color was a strict recessive, both in eosin (the sons) and in red (the daughters).

For the production of F_2, F_1 wild-type females and F_1 eosin star dichaete males were mated together. The first point observed in F_2 was that the gene produced no visible effect by itself, for in that half of the offspring that were not-eosin none of the

eyes were of other than the normal red of the wild-type. On the other hand, among the eosin-eyed offspring about a quarter were of the light color, and this without noticeable sex limitation, that is, the females and males were diluted to the same proportionate extent. On analyzing the distribution of the cream with respect to the second chromosome dominant star and the third chromosome dominant dichaete, it was seen that the gene for the cream is contained in the second chromosome, for the cream appeared only in the flies that were not star, all the flies being either star or cream and none being both star and cream or neither, which condition is in accord with expectation from the lack of crossing over in the male. The 2 : 2 : 0 : 0 ratio observed

TABLE 26

The F_2 offspring given by F_1 wild-type females and F_1 eosin star dichaete males from the outcross of a cream c female to a star dichaete male

1916, 10/20	S′	$\frac{S'}{D'}$	+	D′	$\frac{w^e}{S'}$	$\frac{w^e\ S'}{D'}$	$\frac{(w^e)}{c_{rc}}$	$\frac{(w^e)}{c_{rc}\ D'}$	w^e	$\frac{w^e}{D'}$	$\frac{(we)\ S'}{c_{rc}}$	$\frac{(w^e)\ S'}{c_{rc}\ D'}$
5588[1]	26	21	32	23	23	34	11	11	14	15	0	0
5631[1]	40	32	33	34	40	42	18	16	10	8	0	0
5654	17	37	32	44	40	43	21	17	21	23	0	0
Total.....	83	90	97	101	103	119	50	44	45	46	0	0

[1] Culture 5588 and 5631 produced 'apterous'as the result of a fresh mutation which took place in the cream c stock (Metz, Am. Nat., '14, pp. 675-692).

in the case of star and cream c is in sharp contrast to the 1 : 1 : 1 : 1 ratio in the case of cream c and dichaete in the same experiment. There were as many creams among the dichaetes as among the not-dichaetes, as is expected from the free assortment of genes in different chromosomes.

Up to this point there had been no confusion possible among the various modifiers; for the effects produced had been different in one or more of the following respects: in degree, in color tone, in dominance, in specificity, in interaction with other mutants, in the chromosome concerned, or in the locus within that chromosome. In the case of cream c, there was the chance of confusion, since in tone cream c is not different from cream b, though in

the amount of dilution (degree) there seems to be a very slight difference. The fact that they are both in the same chromosome (II) made essential a further test of their distinctness. The method of linkage (a comparison of the star cream c value with the star cream b value) would be crucial only in case the values were quite different. An easier method, and one which at the same time disposes of the question of allelomorphism is to make a cross between the two types. When this was done (culture 5721) it was found that the F_1 flies were standard eosin in color, as is expected from the cross of two distinct recessives. Evidently cream c is neither cream b nor an allelomorph of cream b, and this is the only case where confusion was possible.

SUMMARY

The significant facts with respect to the origin and the inheritance of these modifiers are summarized in table 27 in which the primary arrangement is according to the order in which the modifiers were discovered. The first four modifiers appeared in rapid succession in the summer and fall of 1913 and were worked out almost simultaneously. In 1914 three more were found,

TABLE 27

Summary of the origin and inheritance of the specific modifiers of eosin

MUTANT	SYMBOL	ORIGIN					CHROMOSOME	LOCUS
		Finder	Date	Stock	Type	Culture		
Cream a	c_{ra}	Bridges	1913, 7/15	Non-disjunction	w or w^e	n 43	—	—
Cream II	c_{rII}	Bridges	1913, 9/15	Lethal 2	W	—	II	—
Dark	—	Bridges	1913, 9/23	Non-disjunction experiments	w or w^e	n 100	—	—
Whiting	—	Bridges	1913, 11/21	Non-disjunction experiments	w or w^e	M67	—	—
Cream III	c_{rIII}	Wallace	1914, 2/27	Lethal 1a	W or w^e	—	III	7.9
Cream b	c_{rb}	Bridges	1914, 3/10	Non-disjunction experiments	W and w^e	82	II	22.5
Pinkish	—	Bridges	1914, 7/27	Eosin	w^e	—	II	106±
Cream c	c_{rc}	Bridges	1916, 7/21	Eosin	w^e	—	II	—

and another in 1916. On various other occasions dilute eosins have been observed whose inheritance has not been followed in detail.[7]

Origin of modifiers. All of the modifiers were first detected in stocks or experiments involving eosin. This fact is not the result of any influence of eosin upon mutation, but has a simple explanation in the fact that the modifiers produce little or no visible effect except when brought into coaction with eosin, and hence they pass undetected no matter how numerous, or what their origin, until this condition is satisfied. Only the last two modifiers, 'pinkish' and 'cream c,' were found in stocks pure for eosin, and in these cases the mutation might have occurred previously and have been incorporated with the eosin stock. The other six modifiers were found in experimental cultures in which only half of the flies were eosin; and in these cases it is usually not possible to say whether the modifier originated in or was introduced through the eosin or the not-eosin half of the experiment. In the case of cream II, there is good evidence that the mutation had occurred in a wild stock and had lurked there undetected until the cross to eosin brought it out of hiding. Cream b seems to have been present in both parent stocks. Creams a and II, dark and whiting, may have arisen in either, though the probability is on the side of the not-eosin parent, since these modifications were likely to have been detected if present in the eosin parent stocks.

The scale of modifications. A graphic representation and comparison of the color differences produced by the modifiers is given in diagram 1.

In constructing this diagram two standards were chosen, namely, the colorless eye produced by the interaction of eosin and 'whiting', and the unmodified eosin. The modified eosins were then spaced along the line connecting these grades in proportion to their intensities of color. That is, homozygous cream II is the lightest of the other modifications and 'dark' is the darkest. Pinkish is the weakest of the dilutors of eosin. Creams

[7] In 1918 two other creams have been found, and the inheritance of one of these presents features of exceptional interest for the chromosome theory of heredity.

c, b, and a are quite similar in color, b and c being especially alike. Cream III is about half-way in color between eosin and white. The grades represent, as near as could be approximated, the mode of each color modification; there is in each case some fluctuation in the direction away from the eosin grade and a greater range in the direction toward eosin. Since the eosin

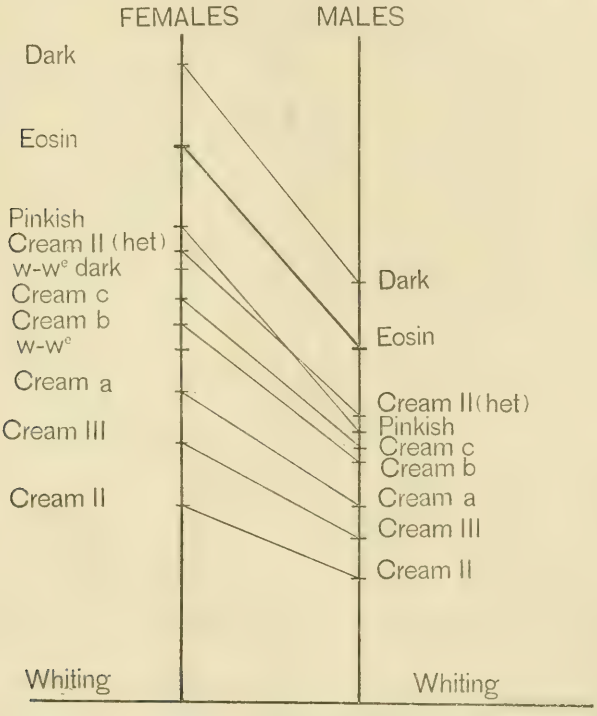

Diagram 1. The scale of modifications of eosin in females and males (modal grades).

male is of a lighter eye color than the eosin female, it became necessary to arrange two such series of grades in order to represent the total range. After the separate distributions of color for the female and the male had been plotted, lines were drawn connecting the corresponding types in the male and female series. The surprising fact was then observed that all but one of the lines, if continued, would intersect the base line at about the

same point. This was a striking revelation of a relationship vaguely realized before—that the modification by each of these genes is proportionately as great in the male as in the female, although the actual modification is much greater in the case of the female.

Sex limitation. The above relation does not hold in the case of pinkish, for the connecting line cuts the base line at a point only about three fourths as distant as the others. The dilution of the pinkish male is proportionally greater than that of the pinkish female. It is just in this respect that eosin itself differs from the other eye colors. The combination of these two 'sex-limited' characters, both of which differ in the same direction, increases the sexual dimorphism of the flies to a striking extent.

Specificity. The primary characteristic of all these modifiers is that their visible effect upon eye color is dependent entirely upon, or is greatly increased by, the coaction of eosin. Of the eight modifiers, six (creams a, II, b, c, dark, and whiting) are entirely dependent, giving no visible effect whatever in the absence of eosin, while pinkish gives a very slight dilution and cream III gives a dilution which is strong enough so that by avoiding certain adverse conditions there is the possibility of using it without the complication of eosin. In the case of pinkish the dilution is so slight that only in about a third of the flies is there any detectable difference. With both cream III and pinkish the ease of separation and the sharpness of the difference is vastly greater in the presence of eosin.

The term 'disproportionate modifier' might perhaps be better for such cases as those of cream III and pinkish. Most of our mutations are what may be called 'general modifiers,' since their effects seem to be independent of one another, and the combined effect is cumulative and roughly proportionate. 'General modifiers' may be represented by the familiar parallelogram, in which the initial condition (wild-type) is one corner, the effect of each gene acting independently is represented by the length and direction of the two adjacent sides, and the combined effect (double mutant type) is the opposite corner (diagram 2, a). In the case of the completely specific modifiers, such as cream II, the length

of the side between the wild-type and (not-eosin) cream II corners is zero, so that the parallelogram becomes reduced to a triangle (diagram 2, b). In the case of pinkish and cream III there is an intermediate condition in which the side between eosin and the double type is disproportionately far greater than the distance between the wild-type and the simple modifier (diagram 2, c). There is another type of disproportionate modification exemplified by eosin sepia which might be called reversed.

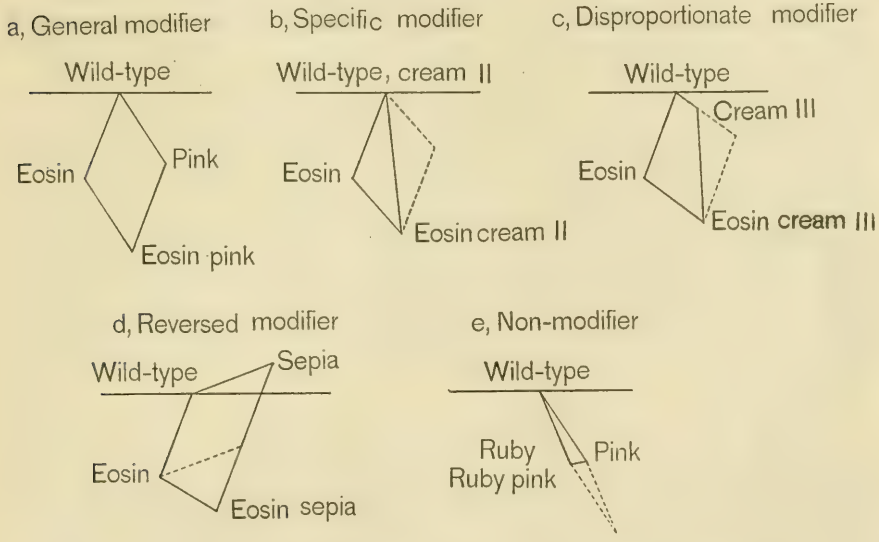

Diagram 2. A graphical representation of some types of modification

Sepia is considerably darker than the wild-type, becoming a deep blue-black in old flies. One might expect that sepia would cause a proportional darkening of eosin so that eosin sepia would be as much darker than eosin as sepia is darker than the wild-type. This is not the condition that actually obtains. The eosin sepia double form is lighter than eosin, about as represented in diagram 2, d. There are several other curious types of disproportionate modification. Thus, for example, ruby (sex-linked) may be described as a 'non-modifier' of pink. The effects of these two genes are in the same direction and of like amount,

but they fail to have a cumulative effect, the double type being practically indistinguishable from either single type instead of being as much lighter as each is lighter than the wild-type (diagram 2, e).

It has been shown for the case of cream II that specificity of a very extreme nature obtains; since cream II is incapable of modifying any of the other mutant eye colors tried in place of eosin. The double recessives (not-eosin) cream II vermilion, and (not-eosin) cream II pink are the same in eye color as simple vermilion and pink, respectively. In the case of whiting the specificity is even more striking; for cherry, an allelomorph of eosin so similar as to be distinguished (in the females) only with great difficulty, is entirely undiluted by the whiting gene.

Tone. Pinkish is aberrant in still a third respect: the other modifications can all be described as grades of dilution of the yellow-pink color of eosin, the lighter grades being especially dilute in the pink component of the color. Pinkish, on the other hand, gives one the impression that the yellow component has faded more than the pink, so that there remains a slightly greater proportion of pink in pinkish than in eosin or in the other modifications.

Dominance. The only one of the modifiers which gives an appreciable effect in heterozygous form is cream II. The amount of dilution of eosin due to heterozygous cream II is of about the same grade that the weakest of the other modifiers, namely, pinkish, gives when homozygous.

Fluctuation. The amount of fluctuation in the eye color of the creams due to differences in age, food, etc., was about the same as that observed in the other eye colors and their combinations. This question is of some practical importance in the making of classifications and is of theoretical interest in connection with the completeness of the seriation obtainable with a relatively few modifiers.

Viability. All of these creams are of excellent viability and the observed ratios are very close approximations to expectation.

Chromosome. Because of the difficulty of putting the modifiers to practical use, it was not at first thought worth while to

determine in which chromosome the gene for a given modifier lies. This information is therefore lacking in the case of cream a, dark, and whiting. Of the five other modifiers, one, cream III, is in the third chromosome, and the other four are in the second. The presence of four modifiers of eosin eye color in the second chromosome is in contrast to, or perhaps supplementary to, the relative fewness of ordinary eye-color mutations in that chromosome. None of the modifiers were sex-linked, but this is probably not of special significance.

Locus. The locus within the second chromosome has been determined for two of the four second chromosome modifiers of eosin, namely, pinkish at about 106, and cream b at 22.5. The locus of cream III has not been found directly because of the presence of C_{III} in the cream III stock. In heterozygous C_{III} there has been found to be 4.2 per cent of crossing over between cream III and dichaete, and 5.4 per cent of crossing over between cream III and ebony. This corresponds to a locus on the normal map of about 3.1 units to the left of dichaete or at about 7.9 units to the right of sepia which is the zero-point of the map of the III chromosome.

DISCUSSION

The facts of the inheritance of these specific modifiers show that each is a definite Mendelian gene on the same footing with the whole body of genes known in Drosophila. Thus, they display clean-cut segregation from their allelomorphs; each is located in and transmitted by a specific chromosome of the Drosophila complex; they give free assortment with genes located in other chromosomes; and with genes located in the same chromosome they show linkage, with crossing over corresponding to a fixed locus. Each of these genes arose by mutation—"a specific change in the hereditary constitution"—by the transformation of the materials of a particular locus into a new form having a different effect upon the developmental processes.

These specific modifications are clear and simple cases of 'multiple genes.' Each is the result of the coaction of a specific

modifying gene (cream a, b, etc.), which by itself produces little or no visible effect, and of a particular gene (eosin) that is necessary as a 'base' or 'differentiator.'

The scale of the modifications of eosin produced by these several modifiers ranges on the one hand to a deep pink darker than eosin, and on the other hand to a pure white. This scale is purely artificial and descriptive, for these modifiers were entirely independent of one another in origin. Furthermore, the order of their occurrence bears only a random relationship to the dark-light seriation.

By judicious combination of several such simple modifiers a multiple heterozygous stock could be obtained which would be amenable to selection and which would offer upon analysis a satisfactory parallel to such a case as that shown by Castle's rats. The first result of selection in such a heterogeneous stock in the direction of lighter forms would be to pick out individuals homozygous for one or more of the modifiers and probably heterozygous for others. These different individuals of course would not necessarily be homozygous for the same factors, and therefore the population might still remain heterogeneous for these factors. Continued selection would result in a greater and greater degree of homozygosity and homogeneity and a consequent slowing down of the speed of the progression of the population in the direction of selection. The grade of the form reached when the population is homozygous and homogeneous would depend on the number and character of the particular modifiers in the initial population.

The fact must not be lost sight of that there is not one iota of evidence to show that either the rate or the direction of the mutation processes that are characteristic of the species are altered by such selection. During the course of any selection experiment modifiers and other mutations should arise at the normal rate. In Drosophila roughly 25 per cent of the mutations found were wing or venation characters, 16 per cent body-color characters, and 20 per cent eye colors. Only eye-color mutations would have any effect upon the progress of our selection. The eye-color mutations found in Drosophila have been roughly in

the proportion of 60 per cent of general or non-specific modifiers of eosin, such as vermilion and pink, 22 per cent specific modifiers of eosin, and 18 per cent allelomorphs of eosin. The proportion in which mutations are found should, of course, be distinguished from the proportion in which mutations arise. In this particular example the difference should be considerable, for since all the flies are eosin, the situation is particularly favorable for the detection of specific and disproportionate modifiers of eosin, and the percentage found should be correspondingly higher than in our general work where eosin flies constitute only a small fraction. It is probable that mutation is very much more frequent than appears, since a great many mutations are of very slight somatic effect and would pass undetected except that certain characters, such as eosin eye color, truncate wings, beaded wings, and a few others, are peculiarly sensitive differentiators for eye-color and wing-shape genes, etc. Mutations capable of affecting eye color, wing shape, etc., are presumably not less frequent of origin in ordinary stocks, such as pink eye or rudimentary wings, but these latter are poor reagents for the detection of mutations. Whether or not a specific modifier should arise during the course of selection depends on the length of the selection, that is, on the number of individuals, as well as on the frequency of that type of mutation.

The previous eye colors (all types) may be roughly classified as 90 per cent dilutors and 10 per cent darkeners of eye color which is about the same proportion as that found among the specific modifiers themselves. Cases like that of eosin sepia show that the ratio of darkeners to dilutors considered from the basis of eosin may be far different from that shown by the primary effects of these same genes (differences from the wild-type). Whatever this normal ratio for eosin is, it should obtain in the selection experiment as well. When mutation in the direction of selection occurs, there should be a jump in the speed of progression, and the final grade should be correspondingly more extreme. However, the modifier might be a darkener, but in that case it would not be selected and consequently would have only negligible effect upon the speed and the resultant grade.

No one can deny that progress under selection is theoretically possible by repeated mutation in a single locus. But to accept that as the actual mode in any particular case demands specific proof. Such a hypothesis should not be considered until it has first been demonstrated that the initial constitution of the stock was such as to require the assumption of further acts of mutation. If a fresh act of mutation is required, then adequate proof must be submitted before it can be accepted that this mutation is in the particular locus favored rather than in one or another of the numerous other possible loci. Our experience with many cases of successful selection in Drosophila has been that even in this form, where the work is aided by such special features as a very small number of chromosomes, by absence of crossing over in the male, and by a knowledge of the initial constitution of the stocks that is not paralleled in other forms, it is often a matter of some difficulty to prove that a particular modification arose during the course of an experiment rather than that it was present in and introduced through one of the parents. How much more difficult, then, would it be to prove in a form where the tests are far less precise and on a relatively small scale that all progress observed during the course of selection is due to the occurrence step by step of fresh mutations? And how much more precarious would it be to affirm that these fresh mutations are all of a single locus, when one remembers that there are probably hundreds or even thousands of loci in which a mutation would have effect upon any given character. The evidence in the case of the creams is diametrically opposed to such a type of explanation, since the creams are manifestly of discontinuous origin in as many distinct loci as there are diverse modifications. The similarity between the creams and such a case as that of the rats must be attributed to the presence in the rats of the only condition met with in the creams, namely, diverse mutation.

In the absence of rigorous tests, no one is justified in assuming that modifiers are not present in any given stock. Not only have the many selections carried out on Drosophila led experimentally to such a conclusion, but it follows from a consideration of the facts of mutation. In the carefully pedigreed experi-

ments of Drosophila we continually observe mutations arising, and are often able to locate the time and place of origin to a particular fly and to within one or a few cell generations. Everyone who believes that mutations are now occurring in his stocks in this manner must also accept the probable occurrence of such mutations in the immediate ancestry of his stocks, since it is not likely that either the rate or tendency of mutation have changed within any period with which we deal. The process of mutation thus gives rise in any stock to a complexity and heterogeneity which is only that 'static condition of diversity' said by Jennings (Jennings, '17) to obtain for any species or kind of organism, such, for example, as the hooded rat. When selection is started in such a stock immediate progress should be expected and this should continue until the stock is homozygous for all the genes capable of modifying the selected character. One cannot be certain that observed progress is due to fresh mutation until selection has been continued long enough so that the initial diversity has been removed. The initial diversity means that mutations had previously occurred in various loci, and the presumption is that during the progress of selection the stock will not cease mutating in diverse loci and henceforth mutate solely in one locus. Muller ('14), MacDowell ('16), Sturtevant ('18), and others have shown that the results obtained with selection in the hooded rats are, aside from one definite new mutation, exactly those expected from simple selection, in a heterogeneous stock of the kind supposed by the Frenchman Jordan, subscribed to by Jennings, and easily explained as the result of previous diverse mutations. It is interesting to observe that the list of cases in which unilocal mutations or contamination may be seriously considered is dwindling with the progress of exact knowledge.

In our opinion, the attempted distinctions between 'saltations,' 'mutations,' and 'variations of slight degree' have led rather to confusion of thought than to clearer thinking. To us these are all a single class, 'mutations,' and the term carries no restrictions of degree, covering the most extreme as well as the slightest detectable inherited variation. Distinctions of degree when ap-

plied to mutations depend largely on circumstances and personalities, and are correspondingly inexact. Thus, students beginning work on Drosophila usually complain that there is almost no difference between pink eye color, for example, and the wild form, so that the classifications are made with considerable uncertainty. With more experience these same workers come to regard this difference as very great and are astonished that they should ever have thought otherwise. Again, some workers are able to distinguish with assurance characters that quite pass the resolving power of other workers seemingly equally experienced.

In the Drosophila work great numbers of mutations have been encountered whose somatic effect may be roughly described as 'slight.' If degree of effect were marked off as abscissae and number of mutations that actually arise as ordinates, it seems probable that the highest point of the curve would be at the least extreme mutations and the curve would fall rapidly and gradually with the most extreme mutations. For most of the problems in which we have been interested precision of classification is essential, and precision is afforded only by the more extreme mutations and by some few of slight degree but definite character. Accordingly, there has been scant mention of the many 'slight' mutations in our accounts, which have dealt in the main with problems in which such characters were of no use as working tools. Likewise, when these mutations are found, they incite little desire to work out the facts concerning their inheritance, chromosome, locus, interactions, etc. However, enough of them have been investigated thoroughly for us to be certain that no departure from normal Mendelian inheritance is involved. Accounts of some of these 'poor' characters have appeared in Carnegie Publication no. 237, which deals with mutants whose locus is in the first or X chromosome. Thus, for example, dot, bow, depressed, green, chrome, and facet may all be fairly described as of 'slight' somatic effect. Accounts of many others will appear in the publications dealing with the second, third, and fourth groups. Even a larger number will be treated in a section on miscellaneous mutations, while scores will never be referred to at all.

It seems probable that the bulk of the mutations that have been permanent contributions to evolution have been those of slight somatic change. Any organism as it now exists must be regarded as a very complex physicochemical machine with delicate adjustments of part to part. Any haphazard change made in this mechanism would almost certainly result in a decrease of efficiency. The greater the extent of the change the more certain the injury, not simply that the particular part is injured more, but also that a disproportionately greater number of adjustments (morphogenetic, physiological, and ecological) are disturbed or destroyed. Only an extremely small proportion of mutations may be expected to improve a part or the interrelation of parts in such a way that the fitness of the whole organism for its available environments is increased. Thus, that length of trunk which is most advantageous for elephants in a given environment might be attained by a single mutation; but in this case it is likely that the trunk would be out of balance with the other structures (physiologically or otherwise) so that all the individuals possessing this feature might become extinct before the appearance of changes in these other parts that would make the trunk a real success. On the other hand, a mutation which made only a slight change in the favorable direction would require less extensive supporting changes in the related structures, besides which it might be of immediate advantage.

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Resumen por el autor, C. H. Danforth.

Pruebas de que las células germinativas están sujetas a selección, basadas en sus potencialidades genéticas

El autor ha emprendido una serie de experimentos para probar la posible existencia de diferencias funcionales en las células germinativas dependientes o relacionadas con la naturaleza de los "determinantes" transportados por el plasma germinativo. La existencia de tales diferencias se demostró por la acción de un agente selectivo actuando sobre las células germinativas. Este agente fué el vapor de alcohol, que al inhalarse por los pulmones se cree que pasa directamente al torrente circulatorio, y desde este a los tejidos germinativos; se administró a gallinas de la constitución genética escogida. Como indice de cualquier selección que pudiera ocurrir la proporción relativa de ciertos rasgos, braquidactilia, polidactilia y color blanco, que aparecieron en la prole producida durante los periodos de tratamiento, se comparó con la proporción de los mismos rasgos producidos durante periodos en los cuales dicho tratamiento fué suspendido. Los resultados obtenidos indican que por lo menos en el caso de ciertos rasgos la selección es posible y que es mas rigurosa cuánto mas severo es el tratamiento.

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EVIDENCE THAT GERM CELLS ARE SUBJECT TO SELECTION ON THE BASIS OF THEIR GENETIC POTENTIALITIES

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INTRODUCTION

A great deal of evidence goes to show that individual differences among adult animals are commonly due to differences in the germ cells from which the animals have developed. In other words, peculiarities in the adult are, in general, indications of peculiarities in the germplasm. It would seem to follow as an obvious corollary of this that the germ cells of a species must be of almost as many classes as are the adult individuals. This being the case, it is perhaps not unreasonable to expect that germ cells should differ more or less widely in their responses to varying conditions and in their ability to function in the production of zygotes. If such an assumption is shown to be justified, it might be invoked to explain several obscure evolutionary tendencies, as well as certain constant departures from normal Mendelian expectations.

As a first step in determining whether or not germ cells carrying factors for a given unit character differ in their physiological responses from germ cells carrying factors for an allelomorph of that character, it seems desirable to work with the sperm or eggs of a heterozygous animal in which the normal proportion of each class of germ cells produced has already been determined. With such an animal all the germ cells are presumably subjected to the same influences, except as those influenced are varied by the experimenter, and consequently a much better control is possible than could be had by comparing results from different homozygous individuals.

The choice of a method of procedure presents a number of difficulties. Attempts to bring special influences to bear on cells within the living body are attended by many uncertainties and, on the other hand, the treatment of suitable germ cells outside the body in most cases requires the development of a special and difficult technique. The desideratum would seem to be the possession of a means of administering definite amounts of reagents to the germ cells without otherwise disturbing their normal environment. Despite the fact that it does not lend itself readily to quantitative investigations, the inhalation method devised by Stockard seems to be the most satisfactory thus far employed. A considerable number of reagents are suitable for use by this method, but ethyl alcohol is the one that has been most completely tested (Stockard, '13; Cole and Davis,'14; Stockard and Papanicolaou, '16; Nice, '17; Pearl, '17[1]). For these reasons ethyl alcohol administered by the inhalation method was employed in the experiments reported in this paper.

Pearl ('17) has interpreted results obtained by himself and others as indicating that inhaled alcohol affects germ cells differently according to their vitality, destroying some, injuring some permanently, and producing no more than a transient effect on others. In the case of the fowl, he believes that practically maximum treatment with alcohol and certain other vapors results in an elimination of weaker germs with a consequent rise in the average vigor of the chicks actually produced. In other words, treating the parent with alcohol vapor results in a selection of germs in favor of those which produce the most vigorous young. It was not shown in his experiments that any characteristics other than those dependent on general vigor were affected by the treatment, the Mendelian ratios for the few characters involved being essentially the same in both experiment and control.

At the time when Pearl's papers appeared the writer was just concluding a breeding experiment with poultry in which several

[1] An extended list of titles relating to the whole subject may be found in the paper by Pearl, '17.

Mendelian characters were being investigated. Since some of these characters were not represented in Pearl's material, and the writer was at the time casting about for a method to test for germ-cell selection, it seemed worth while to continue breeding from the same birds with former conditions maintained except for the introduction of alcohol treatment. The work was planned in no sense as a repetition of Pearl's experiments, but rather as an attempt to extend his methods to a few characters not known to be directly dependent upon inherent vitality. The writer takes this opportunity of acknowledging his indebtedness to the valuable and suggestive paper mentioned above.

The first experiment, 1, was made in the spring of 1917 with results that seemed to point to a selective action of the alcohol vapor. The matter is one of such importance, however, that a repetition of the work seemed desirable before publication of the data. Consequently three further experiments, 2, 3, and 4, were carried out in the spring of 1918. As will appear below, the results obtained in the latter work proved to be essentially consistent with the findings in the first experiment, which fact seems to justify their publication.

GENERAL NATURE OF THE EXPERIMENTS

In each experiment a cross was made between normal, pure-bred stock on the one hand and hybrid, heterozygous birds on the other. The hybrid individuals were subjected for certain periods to two daily treatments with alcohol vapor, during which time as far as possible all eggs laid were incubated (table 2). Since the original purpose of the work was to test the possibility of selecting germ cells rather than of modifying them through influences brought to bear on the soma, each experiment is brief and intensive.

A control was obtained in each case by saving the eggs from the same flock, kept under as nearly identical conditions as possible, during a period before or after the alcohol experiment. All eggs that did not hatch were opened and the character of

the contents recorded. Since the eggs were candled frequently many embryos were obtained only a few days after death and consequently in a good state of preservation. Very few of the dead embryos were found to be too macerated to afford the desired data. Some living embryos were purposely taken. For the sake of brevity, data obtained from eggs laid during the periods of alcohol treatment will be referred to as A, those from eggs laid in the control periods as C. Each experiment, therefore, has an A and a C subdivision. In experiments 1, 2, and 3 the heterozygous individuals were males, in 4 they were females.

The characteristics tested for their possible response to alcohol vapor were brachydactyly, polydactyly, and color. The presence or absence of booting (feathers on the tarsi) and the apparent form of the comb were also recorded, but the former has been found to be only another manifestation of the factor which produces brachydactyly, while chicks that have been allowed to develop have shown that comb form cannot, in the material used, be accurately determined in embryos and young specimens. Sex was not recorded. The nature of the peculiarities under investigation may be briefly indicated.

Brachydactyly is a condition described by Danforth ('19) which manifests itself in a more or less pronounced shortening of digit IV of the foot. This shortening involves the length, and in extreme cases the number, of bones in the digit. Nearly all brachydactyl birds are booted, but there is a small percentage that has unfeathered tarsi. Conversely, there is an occasional individual with feather tarsi which is not brachydactyl. Breeding experiments have seemed to establish the fact that these conditions are interchangeable from the point of view of heredity or, in other words, that they merely represent different expressions of one and the same underlying cause. Consequently, a single term may be used to cover all of these phaenotypic manifestations, although it must be borne in mind that in a certain small number of cases the word 'brachydactyl' used in this sense is not strictly literal. Brachydactyly may be recognized after about the tenth day of incubation. The

index of brachydactyly[2] was computed for all chicks that hatched and each booted individual was assigned to one of three grades based on the number of feathers present on the shanks and toes.

Polydactyly, a well-known condition, seems to be the final manifestation of an early disturbance in the developing rudiment of digit I of the foot. The character is quite variable, ranging, in the present material, from a single enlarged hallux to a condition in which the hallux is replaced by two digits with even the occasional indication of a third. The cases may be arbitrarily grouped into three grades, dependent upon the degree to which the peculiarity manifests itself. Rarely polydactyly was found to extend to the wings. The term as here used is a misnomer to the extent that a certain number of individuals which clearly manifest the fundamental character in question really have only the normal number of toes. Polydactyly can be recognized with certainty and probably in all cases after about the seventh day of incubation.

Color in the present paper refers to only two grades, 'black' and 'white'. While the pure-bred birds used were either clear snowy white (Leghorns) or uniform glossy black (Minorcas), a large percentage of the young of mixed ancestry showed some color indication of their hybrid origin. This was particularly true of the white chicks, many of which had one or more small spots of black down. By 'white' may be understood pure white down or feathers or white plumage with only a little black pigment in the form of a few dark spots. 'Black' covers all other shades, even though some individuals classified as black subsequently turned out to be barred or mottled. From the twelfth day of incubation (or even earlier) every chick could be put unhesitatingly in one or the other of these categories.

[2] The index of brachydactyly is an arbitrary value obtained by dividing the sum of the lengths of the two fourth toes by the sum of the lengths of the two second toes. With brachydactyl chicks this gives a value equal to 1 or less, while with normal chicks the value obtained is more than 1. The division is carried to the second decimal place and the quotient then multiplied by 100 to eliminate fractions.

METHOD OF GIVING THE ALCOHOL TREATMENT

Alcohol was administered by the inhalation method devised by Stockard ('13) in his work with guinea-pigs and subsequently employed by Pearl ('17) in the experiments with poultry. Pearl's technique was followed in its essentials, except that owing to the conditions of the experiments it was possible to use a somewhat simpler inhalation chamber. For experiments 1, 2 and 3 a glazed earthenware crock 20 inches high and 15 inches in diameter, with a measured capacity of 1.98 cubic feet, or about 56 liters, was fitted with a tight cover and a galvanized-iron false bottom. The false bottom was perforated by seventy-three $\frac{5}{8}$-inch round holes and raised on legs $2\frac{1}{2}$ inches high. The cover was made of thick matched boards subsequently soaked in paraffin and padded along the surface of contact with the crock. A window 8 inches square was cut out of the center and covered by a piece of glass set in paraffin. When a treatment was to be given, the false bottom was tipped on edge and pieces of cotton soaked in 95 per cent ethyl alcohol were placed in the crock. A little additional alcohol was poured in, the false bottom replaced and the cover fitted over the top. At the end of from fifteen to twenty minutes, when the atmosphere was found to be saturated with alcohol vapor, the bird was quickly placed inside on the false bottom, the cover being raised as little, and for as short a time, as possible. Although frequent tests failed to reveal any odor of alcohol on the outside, the whole chamber was generally covered by heavy cloths except when observations were being made through the window. Between each two treatments the crock was cleaned and aired.

From 60 cc. to 70 cc. of 95 per cent alcohol were used for each treatment. The amount that vaporized was roughly determined in the following manner. Fresh cotton was soaked in alcohol and then squeezed as dry as possible; 66 cc. of alcohol were then poured over the cotton and on the bottom of the crock. At the end of an hour and thirty minutes, during which time a treatment was given, the alcohol on the bottom was sopped up and the pieces of cotton squeezed as dry as before.

In this way it was possible to recover 42 cc., leaving a balance of 24 cc., as the approximate amount that had evaporated. The absolute alcohol vaporized into the tank therefore probably ranged between 20 cc., and 25 cc. for treatments lasting about an hour. There was always a rise in temperature amounting to about 3.5°C. during an hour treatment (e.g., on May 21, from 26° to 29.5°), and this of course facilitated evaporation of the alcohol. The treatments were usually begun about 9 A.M. and 5 P.M. Their duration varied considerably, as will be noted below.

With the hens used in experiment 4 a slightly different container was employed for administering the alcohol. Enameled specimen jars about 15 inches square and approximately 12 inches high, with close-fitting covers, were found to have a capacity of 1.6 cubic feet. These jars were used as inhalation chambers, and one or two hens placed in each after an excess of alcohol had been allowed to evaporate in them for from twenty to thirty minutes.

REACTIONS OF THE ALCOHOLIZED FOWLS

The degree of alcoholization obtained is perhaps best indicated by the observed responses of the birds to this treatment. While a certain amount of resistance or accommodation was acquired as the treatment progressed, it was apparent that the males were throughout less affected than were the females. The alcohol absorbed on each occasion was sufficient to cause at least a mild degree of intoxication. Since the reactions were very constant, the details of a single treatment may be given as illustrative of the typical behavior of one of the males following the inhalation of alcohol vapor.

On May 29, 1917, the inhalation chamber was prepared as usual and male no. 8 treated from 10 A.M. to 11.15 A.M. During the whole time he sat on the false bottom turning his head only when disturbed by objects seen through the window. When undisturbed the front of the head rested against the side of the jar. The nictitating membranes passed over the eyes at the rate of thirty times per minute. Notes on respiration were made as follows:

Time	*Rate*	*Remarks*
10.15	40 per minute	Shallow.
10.25	25 per minute	Deeper.
10.50	22 per minute	Deep, regular.
11.15	22 per minute	Deep, but irregular.

At the end of the treatment the bird was breathing noisily and with difficulty. The mouth was open.

11.15. Removed from the tank. Quite passive. Did not struggle.

11.17. Returned to the breeding pen. Stepped very high, but not unsteadily. Showed indications of hyperexcitability. Shied at a feather and jumped out of the way of a chick that approached from behind. The labored breathing improved rapidly.

11.20. Picked up pieces of grass and called hens. Very gallant.

11.22. Crowed repeatedly but abnormally.

11.25. Ate heartily when the flock was fed.

This behavior was quite characteristic of the males following both morning and afternoon treatments. The ability to crow was always affected. Occasionally one was unsteady on his feet and they frequently misjudged distances in attempting to jump or fly. Repeated rubbing of the eyes on the back was characteristic, but despite the severity of the treatment, no whitening of the conjunctiva was noted.

As a control for the above-described behavior, on the afternoon of the same day the inhalation chamber was again thoroughly cleaned and aired and the same male treated exactly as before except that no alcohol was used. He was kept in the chamber from 3.10 to 3.45 P.M. During the first half of this period he sat quietly on the bottom of the container, but during the latter half he stood, possibly owing to the gradual exhaustion of oxygen. Respiration fell from 42 to 30 per minute. The moment the cover was removed from the tank he made violent attempts to escape, struggling and squawking. (Nothing of this sort ever happened following an actual treatment.) Returned to the breeding pen he displayed none of the customary excitability, high-stepping and gallantry towards the hens. After a regular alcohol treatment given an hour later he again showed the usual reactions indicative of intoxication.

It will be seen from the foregoing that the immediate physiological effect of the alcohol upon the males, while unmis-

takable, was nevertheless remarkably slight considering the strength of the treatment. It should be noted in passing that there is the possibility of a mild degree of asphyxiation, but this probably is of no importance in the present connection.

The five hens used in experiment 4 were affected much more severely. They were commonly completely overcome and entirely unable to stand for several minutes after being released from the inhalation chamber. Staggering, which was rarely noticeable in the males, was of regular occurrence with these females. Despite precautions taken to the contrary, all but one of them ultimately died from the effects of the treatment. The one remaining individual developed, or had inherently, a considerable power of resistance.

Aside from the immediate but transient effects of the alcohol, nothing was noted except a slight loss in weight. For example, in the fifty-seven days from February 9 to April 7 no. 28 (treated), with an initial weight of 1803 grams lost 133 grams, while in the next fifty-seven days no. 28 (untreated) gained 160 grams, and no. 27 (treated) fell from 1825 grams to 1695 grams, a loss of 130 grams.

DESCRIPTION OF THE EXPERIMENTS

The method of administering the alcohol and the general character of the obvious physiological responses have already been indicated. It remains only to outline the special features of the individual experiments, of which a condensed summary is given in tables 1 and 2. The control period preceded the period of treatment in experiments 1, 2 and 4, but in experiment 3 this relation was reversed. The surroundings and care of all the flocks during both A and C periods were made as constant as possible. Eggs were put in the incubator in all cases, except 1–C, twice weekly and on definite days, so that no egg had been laid more than four days when incubation commenced.

Experiment 1. (February 15, 1917 to June 2, 1917). The heterozygous parent (male no. 8) was raised at the laboratory from mongrel ancestors. His mother (no. 6), was a monster of the

Pygopagus parasiticus type, which, however, produced only normal young. She was a small hen with a rose comb and the plumage of a barred Plymouth Rock. She was not brachydactyl, polydactyl, nor booted. The father (no. 5) was white with a pea comb and unusually heavy feathering on the neck, suggesting Asiatic blood. He was of moderate size, polydactyl, and booted. This is all that can be said of the ancestry of no. 8, the individual which supplied the traits studied in this paper, but the nature of the experiments is such that a more complete pedigree would be of little additional value. Male no. 8, was hatched in 1914. His coloring was approximately that of a barred Plymouth Rock, but with the breast somewhat spotted and with more or less white in the hackle, saddle feathers,

TABLE 1

Comparative statement of the conditions of each experiment

EXPERIMENT NUMBER	DURATION IN DAYS		DAILY DOSAGE (PART A)		
	Part A	Part C	Maximum	Minimum	Average
1	36	63	2 h. 25 m.	1 h. 30 m.	1 h. 45 m.
2	65	34	2 h. 37 m.	2 h. 0 m.[1]	2 h. 13 m.
3	58	63	4 h. 0 m.	2 h. 0 m.	2 h. 27 m.
4	43	64	2 h. 42 m.	1 h. 8 m.	2 h. 5 m.

[1] This is exclusive of one day when, in the absence of the writer, only a single alcohol treatment was given.

and tail. His comb was large and corrugated, intermediate between rose and walnut. He was polydactyl, grade 1 (left foot only); booted, grade 2, and with a brachdactyly index of 88. In view both of his ancestry and his descendants, it is clearly apparent that this bird was heterozygous for brachydactyly and for polydactyly.

The homozygous parents were thirteen pure bred white Leghorn hens of a standard strain. There can be little doubt as to the purity of this stock, especially as regards the characters under investigation.

In this experiment it was to be expected that the germ cells produced by the females would all be of the same class, while those produced by the male would fall into several classes

depending on whether or not they contained determiners for brachydactyly, polydactyly, or both. Such a situation supplies the necessary conditions for a test of germinal selection. Since Leghorn white is dominant, and all germ cells of the females necessarily carried determiners for this trait, no critical data bearing on color selection were to have been expected from this experiment.

TABLE 2

General summary of data from the four experiments

EXPERIMENT		EGGS			EMBRYOS		CHICKS
Number	Part	Number laid	Incubated	Non-fertile	Killed as embryos	Died in the shell	Number hatched
1	A	150	150	5	39	51	55
	C	362	300	34[1]	108	72	86
2	A	191	180	2	11	153	14
	C	106	104	1	19	69	15
3	A	151	150	35	14	60	41
	C	166	155	49	12	60	34
4	A	39	39	0	6	13	20
	C	151	150	2	45	76	27
Totals: A and C separately	A	531	519	42	70	277	130
	C	785	709	86[1]	184	277	162
Totals: A and C added	A + C	1316	1228	128[1]	254	554	292

[1] As explained in the text, this figure is probably too large, owing to the method of recording in 1–C.

C. The control part of the experiment, running from February 15 to April 18, was not originally intended for this purpose, but rather to supply data for the study of the normal heredity and embryology of several traits, including those considered here. Consequently some of the data, especially those relative to fertility, were not entered in strict accordance with the form adopted in the other experiments. However, this set of data is easily comparable with the other sets and, except for fertility, undoubtedly furnishes a reliable control. Of the 362 eggs laid

only 300 were incubated. The 62 unused eggs were discarded from time to time in small numbers whenever the capacity of the incubator was exceeded, the incubator always being filled from the most recently laid eggs. This method leaves no room for unconscious selection as to size, shape, etc.

A. The alcohol treatment began on April 27 and continued until June 1. Beginning May 1, there were two daily treatments averaging a little over an hour in the morning and half an hour in the afternoon. In this period of thirty-six days male no. 8 was kept in the alcohol vapor a total of 63 hours and 11 minutes (table 1).

Eggs were saved from May 3 to June 2. One hundred and fifty were laid, all of which were used (table 2).

Experiment 2. (March 6, 1918, to June 19, 1918). The heterozygous parent in this case was a male (no. 27) hatched in March, 1917, from an egg used in experiment 1-C. He had the general bearing and many of the characteristics of a Leghorn. His color was white, his comb large and walnut-rose. He was polydactyl, grade 3; booted, grade 2, and had an index of brachydactyly equal to 75. It will be apparent by reference to experiment 1 that this bird was heterozygous for the three dominant characteristics with which we are concerned, i.e., brachydactyly (including booting), polydactyly, and white color.

The homozygous parents were six single-combed black Minorca hens purchased from a local dealer who gave assurance that they were pure bred and of a stable strain. Their somatic appearance as well as their racial purity showed them to be homozygous for the absence of the three above-mentioned traits, or, in other words, they exhibited the corresponding recessive characters, normal length of toes, normal number of toes, and black color.

In this experiment, therefore, all the germ cells produced by the females were necessarily of one class, that tending to give black chicks with normal number and length of toes, whereas the germ cells of the male were expected to fall into eight classes, viz.:

Those tending to produce chicks that were

1. Brachydactyl, polydactyl, white.
2. Brachydactyl, polydactyl, black.
3. Brachydactyl, not polydactyl, white.
4. Brachydactyl, not polydactyl, black.
5. Not brachydactyl, polydactyl, white.
6. Not brachydactyl, polydactyl, black.
7. Not brachydactyl, not polydactyl, white.
8. Not brachydactyl, not polydactyl, black.

It will be apparent that by the inspection of any chick it could be determined at once to which of these eight classes the sperm cell involved in its production had belonged.

C. The hens began laying on March 6 and eggs were saved for the control experiment from that date to April 8, during which period 106 eggs were produced (table 2). Two of these were accidentally cracked and therefore discarded. One, laid April 3, was non-fertile.

A. The period of alcohol treatment followed immediately upon the control period. The first treatments, of 1 hour and 2 minutes and 1 hour and 4 minutes, were given on April 8, and the final treatment on June 11. This bird was kept in the inhalation chamber for two periods of at least an hour each every day except May 5, when in the absence of the writer an attendant misunderstanding directions gave only a single one-hour treatment. In the period of sixty-five days no. 27 was kept in the alcohol vapor for a total of 143 hours and 56 minutes. He showed the usual responses: hyperexcitability, occasional weakness in the legs, and crowing ability invariably impaired for many minutes. He was removed from the breeding pen on June 12.

Eggs were saved from April 9 to June 19. During this period 191 were laid, of which eleven, unfortunately, were lost through breakage or otherwise (table 2). The two recorded as non-fertile were laid on April 21 and June 14, respectively.

Experiment 3. (February 9, 1918, to June 12, 1918). The heterozygous parent (no. 28) hatched in June, 1917, from an egg used in experiment 1-A. He was very similar to male no. 27 described above; in fact, these two males were selected

because they were more nearly alike than any other two raised in the 1917 flock. They were at least half-brothers, possibly full-brothers. It may be noted in passing that no. 27 was produced before the father had been alcoholized, no. 28 after the alcohol treatment had begun. No. 28 was white with a large walnut-rose comb. He was polydactyl, grade 3+, showing a trace of a sixth toe on the left foot; booted, grade 2, and with an index of brachydactyly equal to 70. It may be observed that brachydactyly and polydactyly were more pronounced in this specimen than in any of the other individuals used in the four experiments.

The homozygous parents were six black Minorca hens similar to those used in experiment 2 and from the same source. In so far as the stock was concerned, the material for experiments 2 and 3 was as nearly identical as it was possible to make it. The two flocks were kept in the same room separated from each other only by a wire partition from floor to ceiling.

A. Alcohol treatment of no. 28 began on February 9 with two one-hour periods and continued till April 7. The length of the treatments gradually, but rather irregularly, increased till on April 7 two periods of two hours each were administered. Throughout the whole time this bird was much more severely affected than either of the other males, even when the treatments were of the same intensity. 'Weak', 'aimless,' 'walked in circles,' etc., are among the notations. As another indication of the somewhat different reaction of this bird it may be mentioned that no. 8, no. 27, and the alcoholized hens showed a tendency not to defecate during the treatments, at least after the first fews days. This was not the case with no. 27. No. 8 defecated only once during the whole period and no. 27 only three times, while no. 28 defecated thirty-six times. During a period of fifty-eight days he was kept in the alcohol vapor for a total of 141 hours and 32 minutes (table 1).

The hens began laying February 19, and eggs were saved from that date till April 8, during which time 151 were produced. Of these, one laid February 26 was cracked, the other 150 were incubated (table 2).

C. The control period followed immediately, extending from April 9 to June 12, during which time 166 eggs were laid, eleven of which were lost for purposes of the experiment. The data relative to the remaining 155 are given in table 2.

Experiment 4. (February 20, 1918, to June 12, 1918). The heterozygous parents were five hens (nos. 21, 22, 23, 24, and 26) from the 1917 chicks, and sisters or half-sisters of males nos. 27 and 28. Unfortunately, they were not uniform in type. All were white and all were brachydactyl and booted, but they varied in comb form and in polydactyly. Nos. 21, 24, and 26 were derived from 1-C, nos. 22 and 23 from 1-A. Nos. 23 and 24 were polydactyl, the others were not. The average index of brachydactyly was 91.

The homozygous parent was an exceptionally fine black Minorca cockerel from the same source as the hens used in experiments 2 and 3.

Experiment 4 is a reciprocal of experiments 2 and 3. In this case the male produced only one class of germ cells, while the polydactyl females should have produced the eight classes mentioned above and the non-polydactyl hens the classes numbered 3, 4, 7, and 8. Since individual records were not kept, this experiment yields critical data for only brachydactyly and color.

C. For the control, eggs were saved from February 20 to April 24; 151 were laid, of which 150 were incubated (table 2).

A. Alcohol treatment administered as described in an earlier paragraph was begun on April 27. It was planned to give about three hours a day, but this proved to be more than the hens could stand, and individual treatments were frequently cut down to less than an hour. May 5, by an error, only one treatment was given and on May 31, the morning treatment having been later than usual, it was not thought safe to risk an afternoon treatment. May 7, no. 24, which had recently laid, died immediately after being removed from the inhalation chamber. May 22, no. 22, died in the chamber, and a few days later no. 26 was overcome beyond recovery. Finally, on June 8, no. 23, which had been saved with difficulty on two previous

occasions, was lost. With only one hen left, the experiment was discontinued after the morning treatment of that day. Eggs were saved till June 12, the last four being laid by the sole survivor, no. 21.

Of the six hens originally intended for this work one (no. 25) died February 20 at the beginning of the control experiment and four others were lost as a result of alcohol treatment. While an adequate number of eggs had been secured for C, there were, owing to these accidents, only thirty-nine available for A (table 2). These were all incubated and yielded enough data to justify the inclusion of this, the least satisfactory of the four experiments.

RESULTS OF THE EXPERIMENTS

In the fourth column of table 2 is shown the number of eggs incubated in each subdivision of the experiments. In the following columns the results from these eggs are indicated. Numbers in the fifth column, headed 'non-fertile,' refer to eggs in which no development whatever took place. At the end of three weeks of incubation such eggs were clear and full with a firm yolk; indeed on being broken they presented a more attractive appearance than the average 'store' egg. Only such eggs were counted as non-fertile, except in the case of 1-C where the data were originally secured for another purpose. Here, in a column headed 'infertile or dying during the first few hours,' were entered data that did not differentiate betweeen early death and actual infertility. The other figures in this column, those for 1-A and for 2, 3, and 4, are believed to be strictly accurate.

The sixth column indicating embryos killed needs a word of explanation. The capacity of the incubators was not at all times adequate to care for the available eggs. In consequence, since the necessary data can be secured as readily from a fifteen-day embryo as from a hatched chick, many eggs were opened between the fifteenth and twenty-first days. A few additional embryos were taken in earlier stages.

The next column, 'died in the shell,' shows the number of embryos that failed to hatch. This included embryos of from the first to the twenty-first day, but a considerable number of them represent late stages, many having pipped without being able to escape from the shell. Since fresh eggs were put in one or the other of the incubators every day, the temperature could not be varied to meet the requirements of embryos in late stages, and in consequence many chicks which were doubtless originally strong did not hatch. It will be appreciated that the purpose of these experiments was not primarily to produce viable chicks nor to test the vitality of eggs, but rather to test the transmission of certain traits. To that end the main effort was concentrated on bringing as many embryos as possible to a stage where their peculiarities could be determined, the number that actually hatched (last column) being a mere incident.

As indicated in table 2, a total of 1228 eggs were used, of which 1100 proved fertile. From these 1100 fertile eggs there were obtained in the manner just explained 808 embryos and 292 chicks. One hundred and ninety-four of the embryos died before the end of the seventh day and were therefore useless for present purposes. This leaves 906 embryos and chicks which yielded data of value. For all of these the presence or absence of polydactyly was recorded. Eight hundred and thirty-three of them reached at least the tenth day and furnished data on brachydactyly. Finally, 721 developed sufficient down so that their color could be determined. The distribution of these traits in the subdivisions of the several experiments is set forth in table 3.[3]

Table 3 summarizes all the pertinent data and calls for only a brief explanation. It will be understood that the sum of the

[3] It seems unnecessary to extend this paper by the inclusion of the detailed protocols which would fill a number of pages. Data for eggs laid each day were recorded as well as the date and (known or estimated) age of every embryo that failed to hatch. For the chicks that did hatch the measurements of each toe were recorded, the index of brachydactyly determined and the grades of polydactyly and booting estimated. The data will gladly be put at the disposal of anyone who may wish to make use of them.

numbers under 'polydactyly present' and 'polydactyly not present' is always greater than the sum of the numbers under 'brachydactyly present' and 'brachydactyly not present' in the same series because of the fact that polydactyly can be determined at an earlier age than brachydactyly, and in all cases some embryos have died between the two critical stages. In like manner, color cannot be determined till a still later period, and in consequence the numbers under this caption are still further reduced.

TABLE 3

Distribution of characteristics in embryos and chicks

EXPERIMENT		BRACHYDACTYLY		POLYDACTYLY		COLOR	
Number	Part	Present	Not present	Present	Not present	White	Black
1	A	56	64	37	89	88	0
	C	69	107	73	147	129	0
2	A	52	68	48	79	55	55
	C	26	48	30	47	38	35
3	A	52	45	52	48	53	37
	C	38	51	37	53	48	37
4	A	18	14	(2)	(31)	12	16
	C	48	77	(28)	(105)	63	55
Totals: A and C separately	A	178	191	139	247	120[1]	108
	C	181	283	168	352	149[1]	127
Totals: A and C added	A + C	359	474	307	599	269[1]	235

[1] These totals are exclusive of the figures from 1–A and 1–C.

Since in experiment 4 some of the hens used were not heterozygous for polydactyly, the data entered under that head are not comparable to the corresponding data from the other experiments. They are included here, but in brackets.

In table 4 the data presented in table 3 are converted into such a form as to enable more ready comparison between different parts of the experiments. There is some question as to how this could best be done, but the following method was adopted.

First, as to brachydactyly: it was found that upon adding all the control data together there were 464 individuals of which 39 per cent were brachydactyl. Since this is about the frequency of booting in comparable crosses recorded in the literature, it is assumed that 39 per cent represents the normal incidence of brachydactyly under conditions such as obtain in the control experiments. Next the total number of cases in each group is found and the probable error calculated on the assumption that 39 per cent represents the true incidence. This gives the fourth column in the table—headed 'brachydactyl

TABLE 4

Percentage distribution of characteristics. Compare table 3

EXPERIMENT		BRACHYDACTYL		POLYDACTYL		WHITE	
Number	Part	Observed	Expected	Observed	Expected	Observed	Expected
1	A	46.7±3.1	39±3.0	29.4±2.7	36±2.9	100	100
	C	39.2±2.5	39±2.5	33.2±2.1	36±2.2	100	100
2	A	43.3±3.1	39±3.0	37.9±2.9	36±2.9	50.0±3.2	50±3.2
	C	35.1±3.7	39±3.8	39.0±3.7	36±3.7	52.1±4.0	50±3.9
3	A	53.6±3.4	39±3.3	52.0±3.4	36±3.3	59.9±3.5	50±3.6
	C	42.7±3.5	39±3.5	40.1±3.5	36±3.4	56.5±3.6	50±3.7
4	A	56.3±5.9	39±5.7			42.8±6.3	50±6.4
	C	38.4±2.9	39±2.9			53.4±3.1	50±3.0
Totals	A	48.2±1.8	39±1.7	36.0±1.7	36±1.7	52.7±2.2	50±2.2
	C	39.0±1.5	39±1.5	36.0±1.6	36±1.6	53.9±2.0	50±2.1

expected.' The values in the third column represent the percentages actually observed in each case with the probable errors calculated for the respective percentages and magnitudes. Thus in 1-A, for example, the chances, as it is generally expressed, are even that in a random sample of this magnitude the percentage of brachydactyly would fall between 36 and 42 (39±3). The observed percentage is 46.7 with a probable error of 3.1, giving a range of from 43.6 to 49.8. In other words, in the hypothetical case the chances are 7.5 : 2.5 that the value would fall below 42, while in the actual case observed the chances are

the same that the true value (to be obtained from an infinite number of chicks produced under identical circumstances) would fall above 43. This may be taken to mean that the chances against the observed discrepancy being simply a chance occurrence are more than 16 : 1. In 1-C on the other hand the correspondence between observed and expected results is extremely close.

Polydactyly and color are treated in the same manner as brachydactyly, it being assumed on the basis of the controls that the normal incidence of polydactyly is 36 per cent, and on the basis of genetic literature that the incidence of white color should be 50 per cent.

DISCUSSION

In the foregoing sections the purpose and conditions of the experiments have been set forth and the data that they yielded have been presented. We may now examine these data and attempt to interpret their significance. The three characteristics especially investigated were brachydactyly, polydactyly, and color. These will be discussed first.

Brachydactyly. Table 4 brings out the fact that in each instance the percentage of brachydactyly in A is considerably in excess of that in C, the differences in the four experiments being 7.5, 8.2, 10.9, and 17.9, respectively. The results of either of these experiments taken separately would point strongly to the conclusion that alcohol is capable of influencing the percentage of brachydactyly, and the fact that all four of them show such close agreement gives strong assurance that such is the case. If alcohol were without effect, the departures from the normal distribution would not be all in the same direction and the sum of the data from the four A's should show a percentage approximating that from the four C's. Such does not prove to be the case. In the C experiments there were 464 individuals of which 39 per cent were brachydactyl, while in the A experiments there were 369 individuals of which 48.2 per cent were brachydactyl. These figures are sufficiently

large and the difference between the two percentages is sufficiently great to warrant the conclusion that treating parents which are heterozygous for brachydactyly with alcohol vapor results in an increase in the number of brachydactyl offspring produced.

It is perhaps significant in this connection that in the first three experiments, those in which the males were treated, the increase in brachydactyly is roughly proportional to the average daily dosage, the greater the dosage (table 1) the higher the percentage produced. In experiment 4, while the actual dosage was less, it was very apparent that the general physiological effects were much greater, and this fact seems to be mirrored in the more pronounced increase in brachydactyly in 4-A.

It is also of interest that in the three experiments in which the males are involved there is a correlation between the magnitudes of the C's and A's. In other words, if the percentage of brachydactyly is relatively high in one part of the experiment it is also relatively high in the other part, and vice versa. For example, in 3-C the percentage is several points higher than for all of the C's combined, and in 3-A a similar condition obtains in reference to the total A percentage. In 2, A and C are both below their respective averages while 1-A is intermediate between 2-A and 3-A, and 1-C likewise intermediate between 2-C and 3-C. This point will be reverted to in a later paragraph.

The conclusion in reference to brachydactyly that seems justified is that *by treating a heterozygous parent with alcohol vapor of sufficient strength the proportion of brachydactyl to normal offspring can be increased.*

Polydactyly. Three experiments are available for the study of polydactyly. The results of these experiments are not uniform, and when the percentages for all the C's and for all the A's are computed there is found to be an exact coincidence. Such a group of data might well serve to illustrate fluctuations of percentile values in individual samples, and the tendency of these, in a sense provisional, values to approximate the true values as the magnitude or number of samples increases. Such

an interpretation here would imply that the alcohol was without effect on the transmission of polydactyly. While this seems to be the most probable conclusion, there are two points brought out in table 4 that deserve attention. One of them is a phenomenon similar to that mentioned in the discussion of brachydactyly, namely, a positive correlation between the percentage magnitudes of the A and C divisions of each experiment. In 1 are found the lowest values, in 2 intermediate values, and in 3 the highest values. This looks very much as if each of the three males had his own peculiar capacity for producing polydactyl offspring.

The other point mentioned concerns experiment 3, where in A the percentage of polydactyl chicks was 52 which is a most unusual percentage in a cross of this sort. Here an effect of the treatment seems to be indicated, and such a supposition is strengthened by the fact that when the treatments were stopped the percentage dropped to forty. It has been pointed out that in the case of brachydactyly there is some evidence that the amount of rise in the percentage is dependent upon the strength of the treatment. It is possible that the same is true of polydactyly, but that the level required to produce results—the threshold—is higher. Experiment 3-A, which had the highest daily dosage and in which the treated male was most affected, seems to have been the only one that was sufficiently rigorous to produce an effect on the percentage to polydactyl chicks.

For polydactyly it may be said by way of conclusion that the evidence is possibly negative, but that there is some indication that when the alcohol treatment of the heterozygous parent is sufficiently intensive the relative number of polydactyl young is increased.

Color. In experiment 1 the female parents were homozygous for the dominant white characteristic of the Leghorns, while the treated male parent was a homozygous dark recessive. Since each parent could produce only one kind of germ cell, there was no chance for selection and the expectation of 100 per cent of white chicks was realized in the 217 individuals whose color was determined. In the other three experiments

the treated birds were all white and consequently, since they were derived from the cross made in experiment 1, heterozygous for color. These individuals should have developed two kinds of germ cell with reference to their color-producing potentialities, thus affording material for selection. Tables 3 and 4 show the results obtained. They do not reveal any obvious effect of the alcohol unless it be in experiment 3, where the percentage in A is considerably above what one would expect. 3-C also shows a high percentage, and when it is recalled that C followed immediately upon A, the probability that the high percentage in both cases is due to a common cause rather than to chance is somewhat increased.

Except in 4-A, where the total number of individuals involved is only 28, the percentage of white chicks does not fall below 50 in any of the six separate experiments, the average being over 53 per cent. This is rather close to expectation, but the constant upward tendency of the white is at least noticeable and suggests a possible inherent superiority of the white producing germ cells.

No very certain conclusion seems warranted in regard to the effect of alcohol treatment upon the transmission of color but in the experiment in which the treatment of the male was most severe there is some indication that the germ cells bearing determiners for the dominant character functioned more commonly.

In reviewing the four experiments, no. 3 will be seen to have yielded the most striking results throughout. In this experiment, not only did the three characters, brachydactyly, polydactyly, and color, show indications of the action of alcohol vapor upon the germ cells, but the fertility of the eggs was also markedly affected (table 2). This latter point is of interest in connection with Pearl's results. One of the most constant features reported in his paper is a regular elevation of the percentage of infertile eggs in his various alcohol series. When the present work was begun similar results were expected, but as will be seen by reference to table 2, they were realized only in experiment 3.

There are several considerations that may serve to explain this discrepancy. Pearl studied the effects of alcohol acting over long periods, while in the present work it was desired to have the treatments extend over the shortest possible periods consistent with securing a statistically sufficient number of eggs. Since Arlitt and Wells ('17) have shown that in the rat testis cells in different stages of spermatogenesis are affected differentially by alcoholic poisoning, it may be surmised that injury done to cells in early stages of gametogenesis may not show any effects in breeding tests for relatively long periods. It is possible on this assumption that Pearl's data were taken mostly after the full effects of the alcohol had been established, while most of my experiments were stopped too soon to get the later results. The data from experiment 3 are in harmony with this supposition, for in C, which followed immediately after A, the percentage of infertile eggs was actually higher than it was during the time when alcohol was being administered.

Another possibility that may be mentioned is differential susceptibility to alcohol. It might be inferred from Pearl's paper that one hour is practically the maximum time that a fowl can live in an atmosphere saturated with alcohol vapor. The males used in experiments 1, 2, and 3 were able to endure much more than that, but of these three males no. 28 showed unmistakable signs of being most severely affected. It may be, therefore, that although stronger dosages were administered, the actual physiological reactions were less except in experiment 3.

That male no. 28 was not naturally infertile is shown by his subsequent history. During July and till the 10th of August he was mated to no. 21, and this hen gave no infertile egg till August 26, sixteen days after having been separated from the male. Aug. 10, he was mated to eight black Minorcas, the remnants of experiments 2 and 3. Eggs from this mating were saved beginning Aug. 19. Despite the fact that during much of the time the weather was unfavorable and the male was moulting, out of the 101 eggs laid only three were infertile, in marked contrast to the more than 27 per cent of non-fertile eggs during the experiment. Since the number of hens in the

original experiment was smaller than that used in the supplementary test, it does not seem likely that there was a dearth of sperm at any time during the experimental periods, but more probable that some of the eggs were entered by sperm too badly injured to develop a pronucleus or at least a viable conjugation nucleus. An egg 'fertilized' by such a sperm would very likely give no other reaction than an egg that had been reached by no sperm whatever, and would be recorded as non-fertile.

Pearl's statements as to the non-effect of alcohol on the transmission of Mendelian characters are clearly meant to apply only to the data he presents, and those data were derived from experiments obviously neither intended nor adapted for the solution of the problem attacked in this paper. Since he dealt with crosses between pure-bred homozygous strains in which all the Mendelian characters were in stable equilibrium, there was no chance for selection except on the basis of such characters as vitality and vigor. With reference to these characters, Pearl found selection to be possible. In the experiments now being reported the material afforded a chance for selection between other traits, in this case Mendelian, and the results are believed to show that here also selection is possible.

The question still remains as to the precise nature of the selection that takes place. Cole and Davis ('14) have produced evidence that with rabbits the sperm of one male may have greater fertilizing capabilities than that of another, even when conditions would seem to be more favorable for the latter. They have also shown that the fertilizing power of the sperm can be influenced by poisons administered through the male soma. But in their work also, homozygous males seem to have been used and no evidence is presented as to whether or not two kinds of sperm produced by the same male could be differentially affected. These and other results obtained by Cole and his colaborators, Stockard's findings, and the conclusions reached by Pearl, all tend to suggest that the effect of poisonous reagents is lethal rather than stimulating, if such is the case, we may assume that a germ cell or nucleus bearing a determiner for brachydactyly is more resistant to the effects of alcohol treatment than one not bearing such a determiner.

On this assumption it might be expected, although it need not necessarily follow, that germ cells carrying determiners for two or more characters of selective value would be more favorably circumstanced than those carrying only one such determiner. The available data is not sufficient to throw much light on this question, but so far as it goes it would seem to indicate that such may be the case. For example, in 3-A the combination brachydactyl-polydactyl-white is represented by sixteen individuals where the expectation is thirteen on the basis of observed percentages and only six on the basis of expected percentages.

The question as to the time in gametogenesis at which the selection takes place must also be left unsettled. The purpose of these experiments was to try to select between mature germ cells, and this seems to have been accomplished, but there is also some indication that the effects of the treatments have persisted for a period greater than the probable life of such cells. Moreover, there is some evidence, too meager perhaps to be given much weight, that ova, or their nuclei, are likewise selected. This may mean that the alcohol is effective as a selective agent as far back as the first maturation division and possibly determines at that time which nucleus will remain in the egg of the female or which will be the more effective sperm produced by the male. In this connection may be recalled the finding of Arlitt and Wells (loc. cit.) that, in the rat at least, the alcohol affects stages of spermatogenesis in the reverse order, attacking late stages first, and early stages last.

There is one interesting by-product of these experiments which should perhaps be further emphasized, the indication of individual idiosyncracies in the transmission of traits. Among breeders a belief in 'prepotency' has occasionally had currency, but the possibility of this supposed phenomenon being real is often discounted by geneticists. When the data for these experiments were being tabulated it was observed with some surprise that they might be interpreted as furnishing evidence of something analogous to prepotency. It would

not be inconsistent with the conclusions of this paper if it should turn out that within certain limits heterozygous individuals may produce functional germ cells of reciprocal classes in different proportions, the average ratio for all individuals being in most cases about 50 : 50, but in brachydactyly, for example, 39 : 61. Such a tendency, unless very marked, would be masked in ordinary breeding experiments since individual departures would be attributed to chance and lost in the totals. The data in the present paper happen to be so arranged that there are eight opportunities to note the correlation between percentages produced by the same male parents in two different tests, and in all of these cases the correlation is positive. This is a matter that seems to be worth investigating and these data suggest a favorable method for attacking the problem. To test the question properly, many matings between pairs or small groups of animals of proper gametic constitution should be made, and the data from these matings broken up into blocks that could be compared with each other and with similar blocks from other matings.

SUMMARY AND CONCLUSIONS

These experiments were planned to test the possibility of selection between the different classes of germ cells produced by a heterozygous parent. For a selective agent alcohol vapor, which, inhaled through the lungs, is believed to pass directly into the circulation and thence to the fluids surrounding the germinal tissues, was administered to fowls of the desired genetic constitution. As an index of any selection that might occur, the relative proportion of certain traits, brachydactyly, polydactyly, and white color, appearing in the offspring produced during periods of treatment was compared with the proportion of those traits produced during control periods. The results indicate that with at least some traits selection is possible and and that it is more rigorous the more severe the treatment.

Since, under certain conditions, here artificially produced, it appears that germ cells with different genetic potentialities

react differently, a possibility of far-reaching importance is suggested: that, even under normal conditions, the genes which determine the genetic potentialities of a germ cell may have a real survival value for that cell and, moreover, that the prevalence of certain traits appearing in the adult may be in the final analysis largely regulated by the advantage or disadvantages that the determiners for such traits confer upon the germ cells in which they chance to be lodged.

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GENETIC STUDIES ON THE MEDITERRANEAN FLOUR-MOTH, EPHESTIA KÜHNIELLA ZELLER

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ONE FIGURE AND TWO PLATES

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I. INTRODUCTION

A. Distribution and taxonomy

The Mediterranean flour-moth, Ephestia Kühniella Zeller, is widely distributed and very destructive to stored cereals. It was noticed in Paris in 1840 and in Constantinople in 1872. An outbreak of the pest occurred at Halle, Germany, in 1877, where it was supposed to have been introduced with some American wheat. The American origin was assumed by European writers for a number of years, but it is probable that the insect was rather widely distributed in Europe for some time, being noticed only when it became especially abundant and troublesome. It was not officially reported in America until 1889 (Canada).

The insect belongs to the subfamily Phycitinae of the large family Pyralidae. Its nearest important relatives are the dried-currant moth, Ephestia cautella Walker, the chocolate moth, Ephestia elutella Hübner, and the Indian-meal moth, Plodia interpunctella Hübner.

In June, 1877, Professor Kühn, of the university at Halle, sent a number of specimens to Zeller whose description appeared in 1879. It is thought advisable to quote the original description of the moth since the taxonomic type agrees with the genetic type as treated in the present paper. Color characters with which this paper is concerned have been placed in italics in Zeller's description quoted below.

Major, alis elongatis, *ant. cinereis, strigis 2 obsoletis dilutioribus, obscurius marginatis:* priore ante medium posita, oblique, subserrata, posteriore superne fracta, margini postico nigro-punctato admota, *puncto venae transversae nigro gemino saepe in strigulam mutato,* umbra subfasciata ab eo introrsus ad dorsum demissa; post. albidis, subhyalinis, ramis venae medianae griseis. ♂ ♀

Grösse der Homeos. nimbella. Kopf und Rückenschild von dem lichten Grau der Vorderflügel. Stirn gerundet. Taster aufgekrümmt, anliegend, hellgrau, an den Seiten des 2. und 3. Gliedes ausser an der Spitze schwärzlich. Sauger stark, auf dem Rücken hellgrau beschuppt. Fühler grau, undeutlich geringelt. Hinterleib heller als der Thorax, mit weisslichem Bauch. Männlicher Analbusch schmutzig weissgelblich; Genitalzangen ansehnlich, länglich löffelförmig, mit gelblichen, verlängerten Schuppen reichlich bekleidet. Legestachel weisslichgelb, lang hervorstehend. Beine grau; Füsse aussen dunkler mit weisslichen Spitzen der Glieder; Hinterschienen zusammengedrückt, am Ende durch Haarschuppen erweitert; vor der Spitze aussen schwärzlich.

Vorderflügel über 5 und bis fast 6‴ lang (bei einem ♀ nur 4‴), gestreckt, mit sanft gebogenem, nach hinten stärker convexem Vorderrand, wegen der Fransen ungefähr rechtwinkliger Spitze und sanft convexem Hinterrand. *Grundfarbe hellgrau ohne andere beigemischte Farbe. Der erste Querstreifen, in der Mitte zwischen Basis und Querader-punkt, ist schräg, etwas nach aussen gebogen, undeutlich sägezähnig, auf der Subdorsale mit scharfem, einspringendem Winkel, heller als die Grundfarbe, auswärts gewöhnlich nur bis zur Medianader breit schwärzlich gesäumt, darunter im Subdorsalwinkel mit einer deutlichen schwarzen Ausfüllung. Die zwei schwarzen, fast senkrecht über einander stehenden Queraderpunkte (der untere grösser und oft längsstrichformig) vereinigen sich oft zu einem oben verdünnten Querstrich;* von dessen unterem Ende oder dem unteren Punkt läuft ein etwas breiter, nicht immer deutlicher, schwarzer Schattenstreif einwärts zur Mitte des Innenrandes. *Der zweite helle Querstreifen, dem Hinterrande näher als der Querader und jenem parallel, macht in seinem obern Drittel einen scharfen, nach aussen offenen Winkel; er ist etwas sägezähnig und auf beiden Seiten, auf der hintern vollständiger als auf der vordern, mit schwarzen Aderstrichen gesäumt. Vor dem mit groben, schwarzen Punkten eingefassten Hinterrande sind die Längsadern oft fast alle schwarz.* Fransen einfarbig hellgrau.

Hinterflügel spitz, weisslich, durchscheinend, mit verloschener, grauer Hinterrandlinie, welche sich um die Spitze bis in den Vorderrand zieht; auch die Medianader mit ihren Aesten ist grau. Basis beim ♂ ohne Haarbusch. Fransen weisslich, an der Wurzel mit feiner, gelblicher Linie durchzogen. Haare des Abdominalrandes oft sehr blassgelblich.

Unterseite der Vorderflügel einfarbig, schimmernd hellgrau, ganz an der Wurzel mit einigen einwärts verlängerten Haarschuppen des Vorderrandes. Hinterflügel weisslich, am ganzen Vorderrand bis zur Subcostale lichtgrau.

Beide Geschlechter sind wenig verschieden; nur ist das ♀ gewöhnlich das schärfer gezeichnete.

In figure *m* is shown a specimen which closely approximates the type as described by Zeller. It is probable that his speci-

mens may have been a little lighter than the average of my own, for it was the transverse light bands (*"strigis 2 obsoletis dilutioribus"* and *"Der erste Querstreifen,. . .heller als die Grundfarbe.... Der zweite helle Querstreifen,"*) rather than the dark, that appeared especially to strike his attention. In general later descriptions emphasize the transverse dark bands. For example, Miss Ormerod ('89) describes the fore-wings as "pale gray with darker transverse markings."

The typical ground color of the fore-wings is gray or ashy (*"ant. cinereis"* and *"Grundfarbe hellgrau ohne andere beigemischte Farbe,"*) in my specimens and according to all descriptions available, although Riley ('89) mistakenly says "in the typical specimens raised by Zeller the ground color is pure yellow or nearly brownish."

I have not been able to correlate with sex any color character such as mentioned by Zeller. The markings of both sexes appear to me to be equally distinct.

Numerous articles concerned chiefly with the economic importance of the moth are referred to in the bibliographies of American economic entomology by Nathan Banks.

The experiments described in this paper have been carried on by aid of a Harrison Research Fellowship of the University of Pennsylvania. My thanks are due to the members of the Zoological Department for their interest and suggestions.

B. Source of the material

The moths used in the experiments belonged to the following stocks:

Bussey stock. On January 28, 1915, five adult moths were placed in a glass jar 4 inches in diameter and 5 inches high with tin cover screwed down tight. The cover must have admitted but slight circulation of air. The jar was half filled with white flour. It was set in a dark closet at the Bussey Institution, Boston, Massachusetts, and was not disturbed until June 2, 1916. At that time there were many insects in all stages of development. It is probable that the culture might have lasted much longer as the flour was by no means exhausted.

Lowell stock. A box of "Cream of Wheat" from Lowell, Massachusetts, was found in July, 1916, to be infested.

Calvert stock. A large tin box of flour heavily infested was given me by Professor Calvert in the fall of 1916.

Washington stock. A culture was obtained from the Bureau of Entomology, Washington, D. C., in June, 1916.

Strain A of Washington stock. A pair of moths was isolated from the Washington stock on July 11, 1916. The female was probably not virgin. A mass culture was made from the progeny.

During July and the first part of August, 1916, the moths were bred and studied at the Marine Biological Laboratory, Woods Hole, Massachusetts. From then until the end of September they were left in mass cultures. The results described below are from matings made at the Zoological Laboratory of the University of Pennsylvania.

C. Technique

Various methods of rearing the moths have been tried. It has been found convenient to use glass candy jars with an inside measurement of $4\frac{1}{2}$ inches in height by 4 inches in diameter. A small amount of cereal is placed in the jar and the etherized moths are set upon this. If progeny are produced, as may be easily determined by the appearance of webs, more cereal is added.

Pupation normally occurs in silken tubes spun in the cereal, but overcrowding or lack of food sometimes causes the caterpillars to wander. The moths emerge and rest upon the glass. They do not fly unless disturbed, so that it is an easy matter to collect them in a shell vial. After several moths have been thus secured they are turned into a wide-mouthed bottle containing ether fumes. The etherized moths may be studied under a binocular and sorted out for recording and pairing.

Once fertile eggs have been obtained from a pair, there is little difficulty in rearing the larvae. The jar has but to be set in a warm, humid place. The adult moths are, however, extremely sensitive to environmental conditions. Mating apparently occurs

at any ordinary temperature or humidity, but the females are very perverse about egg-laying. The proper conditions have not yet been determined, and consequently the results obtained have been a chance selection from a very large number of pairs set. Usually not more than 5 or 10 per cent of the pairs prove fertile, but I have occasionally had as good a ratio as 50, 60, or even 70 per cent. The most frequent condition of infertility is the failure of the female to oviposit. Examination has been made of a large number of such females. The abdomens have been found to be filled with large eggs apparently mature and normal. Another peculiar condition is the failure of the eggs to hatch unless almost all the eggs are laid. It has been found that if the female retains a large proportion of her eggs, the eggs which are laid do not hatch. The pairs have in this case frequently been observed to mate, but it is possible, nevertheless, that the eggs have not been fertilized. Records are being kept of all these conditions and further studies will be made.

If conditions are warm and humid, moths begin eclosing five weeks after the parents have been isolated. Considerable variation obtains in rate of development of the larvae from any one pair, so that moths of one fraternity are sometimes eclosing over a period of one, two or even three months. It is probable that this tends to compensate for the high sterility, for if conditions are not favorable for oviposition at one time they may be at another. There is thus no difficulty in keeping mass cultures. Even extreme reduction in food with consequent reduction in size of the moths does not exterminate them. Despite the extreme sensitiveness of the adults, the species is adapted to tide over very unfavorable conditions.

Since mating takes place as soon as the wings are dry, females are counted as virgin only when found in cultures before a male has emerged.

II. OBSERVATIONS, EXPERIMENTAL DATA, AND CONCLUSIONS

A. Description and origin of variations noted

1. Description of variations. *a.* Color. During the studies at Woods Hole and later the moths were examined for variations. It was soon noticed that there were many minor differences in wing color and pattern and that these appeared to be hereditary. Certain cultures produced moths darker than others, while some had the transverse bands of the wings very well marked. These differences persisted regardless of the nature of the food.

A few color variations were very well marked. The variety shown in figure *n* has been called 'sooty base' or 'sooty.' The base of the primaries is black and the outer margin is much darkened. There is also a decided tendency for the intermediate area to be lighter than in the type. The factor producing this variation, *S*, proves dominant to type.

Another variation tends to darken the mid-area of the primaries, and possibly to lighten the base and outer margin. It has not as yet been studied satisfactorily. In some specimens it is very pronounced, but in others it grades into type. It is of interest because it has an effect the reverse of sooty base. It has been called 'Dark mid-area' or 'Dark.'

A black variation, acting as a simple recessive, *b*, is shown in figure *o*. The upper side of the primaries is black. The upper side of the secondaries is slightly darkened. The under side of the wings is light gray or white as in type, but some black appears along the costal margin. The legs and body are black or gray.

The homozygous sooty black, SS.bb, is shown in figure *p*.

b. Size. Variations in size are probably due to lack of sufficient food, since small moths have come out of certain crowded cultures. When virgin matings of these were made they produced moths of normal size.

c. Leg spines. Two spines occur at the tips of both the middle and the hind tibiae and two occur in the middle of the hind tibiae. They were studied in many hundreds of moths from

mass cultures and individual matings. The number was always constant, and only slight variation occurred in length and divergence.

d. Genitalia. No secondary sexual characters could be found. The sexes may be readily distinguished, however, by the claspers of the male, as shown in figure *b*, and the ovipositor of the female, as shown in figure *e*. Occasionally a moth was found that had peculiar genitalia. Examination showed that these were males in which the claspers were shortened, twisted, or lacking. Internal sexual organs were apparently normal and spermatozoa were present in all.

e. Mouth parts. The adult moths do not feed. The mouth parts consist of the tongue, which is formed by the maxillae; the maxillary palpi, which are small and inconspicuous; and the three-jointed labial palpi, which conceal the maxillary palpi. The normal condition of the mouth parts is shown in figures *c* and *f*. Many variations were observed in the tongue and in the labial palpi. The latter will be referred to hereafter as the palpi.

Figure *a* shows a condition in which the palpi are fused in the median plane. The joints of each are fused with the corresponding joints of the other, so that there results a large flat three-jointed median palpus. The tongue arises from the normal position above the insertion of the palpus and appears to be normal in every way. Its coil is pushed aside by the palpus. Figure *d* shows a condition in which the palpi are asymmetrical. The two terminal joints of the left are lacking. The right is normal. Figure *l* shows a condition in which the palpi are both shortened symmetrically. All sorts of variations in the palpi may occur, due to loss or shortening of the joints.

The tongue may be lacking altogether, as shown in figures *j* and *k*. This variation occurred in some of the mass cultures early in the work. Much variation occurs also in length. The elements of the tongue, maxillae, which are normally united to form a tube, may be separated to any extent. The separation may occur at the tip only or from the tip any distance towards the base. In some cases also the basal or middle part may be

divided while the distal part is normal. Failure of the maxillae to unite is apparently due to malformation.

Figure *g* shows a condition in which the palpi are very small, the tongue is cleft to the base and a short distance from the base the elements diverge laterally. In figure *h* the palpi are normal; the maxillae are separated distally. Occasionally a moth fails to shed the pupal covering of the head. It may otherwise be quite normal. Figure *i* illustrates this, showing a ventral view in which also the maxillae are separated and coiled up at the sides.

Other variations are swellings on the tongue or antennae, straight tongue and scaleless areas on wings.

2. Analysis of original stocks. Summaries of the earlier results will be given in order to show the origin of the variations studied.

a. Bussey stock. Defective mouth parts. Nine non-virgin type females from the Bussey stock, when isolated, produced 841 type, 448 males and 393 females; 6 with defective palpi, 2 males and 4 females; 5 with deformed tongue, 2 males and 3 females; and one male with defective genitalia. Defects of palpi, tongue, and male genitalia, therefore, occur in the Bussey stock.

Twenty virgin females from the Bussey stock were paired with males, either from the Bussey, the Lowell, or the Washington stock. They produced 473 type, 255 males and 218 females; 3 with defective palpi, 1 male and 2 females, and 1 female with cleft tongue. Each of these abnormalities occurred among the offspring of a different mating, so that no significant Mendelian ratio appeared.

b. Lowell stock. Defective mouth parts and sooty base. Ten non-virgin type females from the Lowell stock, when isolated, produced 917 type, 452 males and 465 females; 24 with defective palpi, 13 males and 11 females; 7 with deformed tongue, 4 males and 3 females. Defects in palpi and tongue therefore occur in the Lowell stock as in the Bussey stock.

A pair of type produced 55 type, 25 males and 30 females; and 19 with cleft tongue, 9 males and 10 females. Another similar mating produced 27 type, 17 males and 10 females, and 8 with cleft tongue, 4 males and 4 females. This amounts to

82 type and 27 with cleft tongue, suggesting that the latter may carry a factor acting as a simple recessive.

Among the moths of this stock were also noticed several of the sooty base variety (figure *n*). A pair of these produced 2 sooty, 1 male and 1 female, and 1 type, a female. This indicates that sooty may be dominant to type.

c. Calvert stock. Cleft tongue and sooty base. The moths of the Calvert stock were rather light in color. Some of them had wings with sooty base.

Two non-virgin females produced 101 moths with normal mouth parts and 2 with defective palpi. A sooty male was paired with a type female whose virginity was not certain. There were produced 7 type and 6 sooty. A pair of these type moths produced 9 type and 2 with cleft tongue, suggesting again the recessive character of cleft. The Calvert stock is of interest as it is the source of the factor for sooty base used in later experiments.

d. Washington stock. Defective mouth parts. Sixteen non-virgin females from the Washington stock, when isolated, produced 1523 type, 744 males and 779 females; 11 with defective palpi, 7 males and 4 females; 3 with deformed tongue, 2 males and 1 female, and 1 female with defective palpi and deformed tongue.

Two other non-virgin females were isolated from a fraternity consisting of 44 moths with normal mouth parts. One of these produced 163 type, 85 males and 78 females; 7 with cleft tongue; 5 males and 2 females, and 2 with defective palpi, 1 male and 1 female. The other produced 116 type, 54 males and 62 females, and 40 with cleft tongue, 26 males and 14 females, thus closely approximating the Mendelian three to one ratio. A pair of these moths with cleft tongue produced 2 males and 1 female, all having cleft tongue. This furnishes additional evidence that cleft depends upon a recessive factor.

e. Strain A of Washington stock. Black color and dark mid-area. On October 1, 1916, two males and four non-virgin females of typical color from strain A of Washington stock were isolated in a fresh culture. From December 1 to December 14 there

emerged 105 males and 120 females of typical color, and 1 male, typical in color but with deformed genitalia. There also appeared for the first time 43 black moths, 18 males and 25 females. The factor for black was probably carried as a recessive by the Washington stock.

In later generations bred from this strain there appeared moths with dark mid-area.

B. Tests for the hereditary nature of variations noted

1. Heredity of color variations. a. Black. Summaries of all the results involving black will be given here whether or not other variations entered into the crosses. The recessive nature of black is demonstrated without question.

Fifteen pairs of homozygous gray by black produced 1099 gray. Dominance was complete.

Nineteen pairs of heterozygous gray by black produced 494 gray and 429 black. The deficiency of black below the expected one to one ratio is to be noted.

Three pairs of black produced 114 black indicating that black breeds true.

Fifteen pairs and two mass cultures of heterozygous gray produced 3353 gray to 969 black where 3241.5 to 1080.5 is to be expected. Again the deficiency of black is to be noted.

In cultures segregating gray and black there was considerable variation in ratios. This variation was so extreme in some cases that it is probable that disturbing causes such as lethal factors may have been acting.

b. Dark mid-area. It has not as yet been possible in all cases to separate moths of the dark mid-area variety from type. The gradation appears to be continuous. In some specimens the character is very pronounced. It is carried by black where it may sometimes be seen as a 'ghost pattern.'

A mating of a black male by a dark female and three matings of dark males by black females produced 126 gray and 118 black, which is very close to the one to one ratio expected from heterozygote by recessive. The grays graded from dark to type.

A pair of dark produced 73 gray and 23 black, which is close to the three to one ratio expected from heterozygote by heterozygote. Again the grays showed much gradation between dark and type.

Another pair of dark produced 13 dark in which the character was very pronounced showing no gradation toward type.

A black male paired with a type female produced 14 gray grading from type to dark and 14 black.

Another pairing of black male by type female produced 24 gray, all of which showed the dark mid-area. In these two matings dark was evidently introduced with black.

c. Sooty base. Sooty base acts as a simple dominant to type, but its separation from type is sometimes rendered difficult by the presence of dark mid-area and other variations. This is more fully considered in another section. Only those fraternities in which the distinction from type was clear are here summarized.

Three pairs of homozygous sooty by type produced 430 sooty. Dominance was practically, although not quite, complete.

Two pairs of sooty produced 42 sooty.

Six pairs of heterozygous sooty by type produced 77 sooty and 93 type. The deficiency of sooty below the one to one ratio is to be noted.

Thirty-eight pairs of heterozygous sooty produced 1627 sooty to 615 type where 1681.5 to 560.5 is to be expected. Again the deficiency of sooty is to be noted.

No significant variation in ratios in cultures segregating sooty and type could be found. This is in marked contrast to the condition noted in regard to black.

2. Heredity of oral defects. a. Cleft tongue. The masking of a Mendelian difference by environment. The appearance of cleft tongue is much complicated by environmental conditions. Certain strains never throw cleft, while in others cleft occurs in variable ratios. It has been noticed that fraternities containing moths with cleft tongue show cleft emerging in large numbers at certain times and scarcely at all at others. The cultures have been shifted from humid to comparatively dry conditions and

the appearance of cleft seems to be correlated with drying out. It has also been noticed that when few cleft appeared, the tongues were but slightly cleft.

Six pairs of normal produced 307 normal and 24 cleft, a few cleft appearing in each fraternity. Conditions were rather variable as regards humidity.

Three fraternities from normal parents were reared under dry conditions. They consisted of 55 normal and 19 cleft; 27 normal and 8 cleft, and 116 normal and 40 cleft, respectively. The cleft showed tongues cleft practically to base. Pairings were made of cleft from the last fraternity, but conditions were extremely dry and only one pair produced offspring, 3 moths with tongue cleft to base. Results indicate that we have here a recessive gene which expresses itself only under more or less arid conditions. The three fraternities reared from normal under dry conditions, when summarized, show 198 normal to 67 cleft, very close to 198.75 to 66.25, the monohybrid expectation.

Two pairings of normal by deeply cleft produced under moderately dry conditions 95 and 89 normal, respectively. Moths from the former fraternity produced 262 normal to 51 cleft and moths from the latter fraternity produced 789 normal to 114 cleft. Conditions were somewhat variable during the rearing and eclosion of these F_2 moths. When summarized there were 1051 normal to 165 cleft. The expectation from a monohybrid cross would be 912 to 304, which shows that cleft apparently falls below expectation.

In the later course of the work a very high humidity was maintained. Under these conditions a pairing of a normal by a cleft from the segregating generation summarized above produced 148 normal to 11 cleft. Since one of the parents was cleft and cleft appeared among the progeny we would expect to have a one to one ratio, the ratio from heterozygote by recessive. The deficiency of cleft may be explained by the high humidity. Under these conditions four pairs of cleft by cleft produced 134 normal and only 12 cleft.

The irregular appearance of cleft may then be explained by the effect of environment. Further work is desirable to give added corroboration of this theory.

b. Defective palpi. Defects in the palpi are likewise hereditary, but irregular in appearance.

A pair of moths both of which had defective palpi produced 48 moths with normal palpi.

A pairing of a normal male with a female having both palpi small and a pairing of a male having both palpi small with a normal female produced 189 normal and 12 cleft. No defects in palpi appeared.

A cleft male paired with a normal female produced 142 normal, 77 males and 65 females; 5 cleft, 2 males (1 with defective genitalia) and 3 females; 6 with defective palpi (3 of which had both short), 4 males and 2 females, and 6 with tongue cleft to base and palpi both short, 3 males and 3 females. There were 5 with cleft tongue but normal palpi, 6 with normal tongue but defective palpi, and 6 with cleft tongue and defective palpi. The correlation of defects in palpi with defects in tongue is unusual. Moreover, the shortening of both palpi rather than asymmetry is not the usual condition, but is here seen in 9, at least, of the 12 individuals with defective palpi.

Many large fraternities bred from normal had a very few moths with defective palpi, while many others showed none at all. The heredity of defects in palpi appears to be rather complicated. It has as yet been impossible to correlate the condition with any environmental effect.

C. Tests for linkage of cleft, black, and sooty

Two black females with deeply cleft tongue were selected from the black-producing cultures. One was paired with a gray normal male from the same culture from which she was taken. There were produced 95 gray normal. The pair was recorded as (105). The other black cleft female was paired with a gray sooty moth with normal tongue from the Calvert stock. There were produced 89 gray normal. Records were not kept in reference to sooty. The pair was recorded as (109).

The data given in the remainder of this paper deals with the descendants of pairs (105) (type male by black cleft female) and (109) (sooty male by black cleft female).

1. Free segregation of cleft and black. The F_2 generation from (105) consisted of 204 type, 58 black, 36 cleft, and 15 black cleft. There are 240 gray to 73 black, the expectation being 234 to 78. There is thus no appreciable deficiency of black. There are 262 normal to 51 cleft, the expectation being 234 to 78. There is thus deficiency of cleft. Among the normal there are 204 gray to 58 black, the expectation being 196.5 to 65.5. Among the cleft there are 36 gray to 15 black, the expectation being 38.25 to 12.75. The deviation from expectation is not enough to indicate linkage.

The F_2 generation from (109) consisted of 599 type, 309 males and 290 females; 190 black, 95 males and 95 females; 87 cleft, 41 males and 46 females, and 27 black cleft, 12 males and 15 females. The summary shows 686 gray to 217 black and 789 normal to 114 cleft, the expectation being 677 to 226 in each case. There is obviously a considerable deficiency of cleft, while black agrees closely with expectation. It may be readily observed that there is no evidence of linkage between cleft and black.

Summarizing the F_2 generations from matings (105) and (109), there are 803 gray, 248 black, 123 cleft, and 42 black cleft. There are 926 gray to 290 black and 1051 normal to 165 cleft. The expectation in either case would be 912 to 304. There is clearly, then, a great deficiency of cleft. Among the normal there are 803 gray to 248 black where the expectation is 788 to 263 and among the cleft there are 123 gray to 42 black where the expectation is 124 to 41. There is, then, no evidence of linkage between cleft and black.

2. Free segregation of cleft and sooty. Sooty base showed in a large proport on of the offspring of pair (109), but while very pronounced in some individuals it graded into type in others. This gradation was due in large part at least to the presence of dark mid-area. The moths were classified, however, in a more or less arbitrary manner in relation to this character. The progeny of pair (109) may have been of mixed composition as regards sooty, since the male parent may have been heterozygous. The F_2 generation showed cleft distributed apparently without reference to sooty. If complete linkage occurred between cleft

and sooty and if cleft is recessive as the F_1 generation apparently indicates, no cleft sooty should appear in F_2. If the linkage was partial the ratio of sooty to type should be higher than that of cleft sooty to cleft. Considering only the gray, since black introduces a further complication, which is entirely explained below, there were 249 sooty to 350 type, or 71 per cent; and 34 cleft sooty to 53 cleft, or 68 per cent. The difference between the ratios is not great enough to be significant. Although the classification was admittedly arbitrary as regards sooty, any error would not affect its relation to cleft. It may therefore be concluded that no linkage appears between cleft and sooty.

3. Apparent complications of black and sooty. In the black moths of the F_2 generation from pair (109) accurate separation of sooty and non-sooty was uncertain as in the gray. It has been possible to test for linkage of cleft with sooty and with black. As regards relations between black and sooty, however, complications arise since the character affected is the same, namely, the color of the wings. If the classification were not disturbed by this fact and if no linkage existed, the ratio of sooty to type should equal the ratio of sooty black to black. There are recorded 283 sooty, 403 type, 19 sooty black, and 198 black. This gives 70.2 per cent as the ratio of sooty to type and only 9.6 per cent as the ratio of sooty black to black. It is evident from a comparison of these two ratios that there is either close linkage between sooty and black or else disturbance in dominance. Results recorded below prove that the latter is the case.

D. Analysis of the gradations of sooty base

It will be recalled that the variation called dark mid-area has an effect somewhat the reverse of sooty base. Dark mid-area appeared in many moths of the F_2 generation from (109) and doubtless accounts very largely for the difficulty in distinguishing sooty from type.

A pair of moths in which the primaries had rather dark bases and which were probably sooty with dark mid-area produced 72 sooty, 77 type, and 10 that were intergrading between sooty

and type. The ratio appears like that produced by the crossing of a heterozygous dominant with a recessive but with some difficulty in classifying certain of the individuals. A pair of definitely sooty moths from this fraternity which probably lacked dark mid-area produced 30 definitely sooty and 6 definitely type.

A type male paired with a sooty female produced 25 sooty, 10 males and 15 females; 17 type, 10 males and 7 females, and 6 moths of intermediate appearance, 3 males and 3 females. A pair of these sooty moths produced a fraternity of 28 males and 30 females among which the gradation from sooty to type was so continuous that I found it impossible to make any good separation. A pair of these that showed rather dark wing-base produced a fraternity of 15 males and 14 females showing gradation. A pair of the type moths from the original fraternity produced 52 moths of typical color and these produced in the next generation 105 of similar character. It is possible then to extract type from an intergrading stock.

A pair of type moths produced 36 males and 38 females which showed considerable variability in the pigmentation of the base of the wings. None of them were dark enough to be called sooty, however. A pair of moths having wings with rather dark bases were selected and produced progeny that were classified as type, 10 males and 8 females, and moths with rather dark wing-base, 4 males and 6 females. The gradation was really continuous, but the evidence shows that the tendency toward darkening of the wing-base is hereditary. Such a tendency, occurring in a culture that is segregating sooty as well, would be very confusing. It appears, then, that a hereditary tendency to darken the base of the wing, as well as the variation known as dark mid-area, may tend to obscure the distinction between sooty and type.

In the fraternities discussed below, the distinction between sooty and type was perfectly clear, indicating that the confusing factors were not present.

A sooty male paired with a type sister produced 111 sooty, 53 males and 58 females. Heterozygous F_1 moths when paired produced 134 sooty, 61 males and 73 females, and 70 type, 35 males and 35 females, the expectation being 153 to 51.

A type male paired with a sooty female produced 12 sooty, 6 males and 6 females, and 7 type, 4 males and 3 females. A pair of these sooty produced 26 sooty, 10 males and 16 females, and 9 type, 5 males and 4 females. A sooty male from the same fraternity was paired with one of his type sisters. They produced 16 sooty and 20 type.

A black male paired with a sooty female produced 6 sooty and 8 type showing dominance of gray over black and segregation of sooty and type.

A type male paired with a sooty female produced 11 sooty, 17 type, and 1 black, which is close to expectation as regards sooty, but somewhat aberrant as regards black.

Two cultures of heterozygous sooty produced 124 sooty and 40 type, very close to the expected 3 to 1 ratio.

A sooty male paired with two sooty females produced 42 sooty.

Sooty is then a character which segregates sharply from type when once the complicating factors have been eliminated.

E. Reversal of dominance of sooty by black

A sooty male paired (125) with a black female from the same culture produced 129 sooty, 66 males (one with defective genitalia) and 63 females. The cross may be represented, SS.BB × ss.bb. Six pairs of the sooty progeny, Ss.Bb. produced offspring as shown in table 1.

TABLE 1

COMPOSITION OF CULTURE.	OFFSPRING							
	Sooty		Type		Sooty black		Black	
	♂	♀	♂	♀	♂	♀	♂	♀
1♂ × 1♀	7	9	3	0	0	1	0	3
1♂ × 1♀	18	25	3	8	0	0	4	5
1♂ × 1♀	23	29	10	11	1	2	8	9
1♂ × 1♀	13	20	2	8	4	2	0	3
1♂ × 1♀	15	22	9	6	2	0	4	6
3♂♂×1♀	20	17	6	12	3	2	8	7
Totals..	96	122	33	45	10	7	24	33

Among these 370 moths there are recorded 218 sooty, 78 type, 17 sooty black, and 57 black. There are 296 gray to 74 black, showing slight deficiency of black, the expectation being 277.5 to 92.5. Among the gray the proportion, 218 sooty to 78 type, is very close to expectation, 222 to 74. Among the black however, the ratio is reversed, there being 17 sooty black to 57 black. Apparently black reverses the dominance of sooty. On the basis of this reversal the expectation is 18.5 to 55.5, very close to the numbers actually obtained. Certain black moths have the sooty base appearing as a 'ghost pattern.' This is the heterozygous condition, sS. The moth shown in figure *o* is of this character. It is impossible to separate these from other blacks with any accuracy, but easy to distinguish the homozygote, SS, since the factor S not only blackens the base and outer margins of the wings, but also suppresses pigment in the intermediate area. In the duplex condition of the factor this supression is strong enough to affect the black pigment of the bb moth, as in figure *p*.

A sooty black male of this type, SS.bb, was paired (127) with a type female, ss.BB. There were produced 190 sooty, 80 males and 110 females. Cultures were made from these sooty as shown in table 2.

TABLE 2

COMPOSITION OF CULTURE	OFFSPRING							
	Sooty		Type		Sooty black		Black	
	♂	♀	♂	♀	♂	♀	♂	♀
1♂×1♀	22	23	9	8	1	1	9	7
1♂×1♀	12	19	6	3	5	2	5	7
1♂×1♀	43	39	26	15	2	2	16	24
1♂×1♀	36	39	13	18	2	5	9	9
1♂×1♀	25	28	13	12	5	1	11	12
1♂×4♀♀	124	107	43	38	13	13	26	33
2♂♂×1♀	30	37	14	12	6	0	8	8
1♂×2♀♀	80	90	31	30	11	10	20	18
2♂♂×3♀♀	88	97	29	28	9	8	26	25
1♂×1♀	18	13	9	14	1	1	5	8
1♂×1♀	12	2	1	1	0	1	0	0
1♂×1♀	21	20	6	1	3	0	3	4
1♂×1♀	19	20	8	10	1	0	2	12
Totals....	530	534	208	190	59	44	140	167

Among the 1873 moths there recorded, are 1064 sooty, 398 type, 103 sooty black, and 307 black. There are 1463 gray to 410 black, showing a slight deficiency of black, the expectation being 1405 to 468. Among the gray the proportion, 1064 sooty to 399 type, shows a slight excess of type, the expectation being 1097 to 366. Among the black the ratio, 103 sooty black to 307 black, is remarkably close to 102.5 to 307.5, the expectation with a reversal of dominance.

Summarizing the F_2 generations from pair (125) (SS.BB ♂ × ss.bb ♀) and pair (127) (SS.bb ♂ × ss.BB ♀), we have 1282 sooty, 477 type, 120 sooty black, and 364 black, where the expectation on a 9:3:1:3 basis is 1261.8 : 420.6 : 140.2 : 420.6. The divergence from the expected ratio is largely due to the deficiency of black. There are in all 1759 gray to 484 black, the expectation being 1682 to 561. Considering the gray alone, there are 1282 sooty to 477 type, the expectation being 1319 to 440. Considering the black alone, there are 120 sooty black to 364 black, the expectation being 121 to 363.

A convenient method of expressing dominance in a reversal of this sort is to reverse the relative positions of the symbols placing the small letter before the large. In writing the formula of a phenotype alternative possibilities may be expressed by symbols in parentheses. Thus a moth typical in appearance would be either ss.BB or ss.Bb, expressed ss.BB(b): a sooty would be SS(s).BB(b): a sooty black would necessarily be homozygous, SS.bb: and a black would be ss(S).bb.

Pairings were made of moths from the F_2 or segregating generations from matings (125) and (127). The genetic composition of these moths must be inferred from their appearance and the character of the fraternities produced. The results are of interest, since they corroborate conclusions concerning the heredity of sooty and black.

Each of four pairs of sooty by sooty produced only sooty, 246 in all, showing that each pair must have been SS × SS(s) and BB × BB(b).

Each of four pairs of sooty by sooty black produced only sooty, 275 in all, showing that each pair must have been SS(s).BB × SS.bb.

Two pairs of sooty by sooty black produced 46 sooty and 50 sooty black, showing that each pair must have been SS.Bb × SS.bb.

A pair of sooty produced 56 sooty and 21 sooty black, showing that each parent was SS.Bb.

A sooty black male paired with a black female produced 58 black, showing that the pair was SS.bb × ss.bb. The black progeny, sS.bb showed sooty indistinctly as a 'ghost pattern.'

Two pairs of sooty by sooty produced 29 sooty, 12 type, 1 sooty black, and 10 black. Since sooty, type, and black were produced from each mating, the parents must have been Ss.Bb.

A black male paired with a sooty female produced 6 sooty, 7 type, 3 sooty black, and 4 black. Since both type and sooty black were produced the parents must have been sS.bb and Ss.Bb. The theoretical ratio would be 3 sooty (1 SS.Bb and 2 Ss.Bb), 1 type (ss.Bb), 1 sooty black (SS.bb), 3 black (2 sS.bb and 1 ss.bb).

A sooty male paired with a type female produced 26 sooty, 33 type, and 30 black. Since both type and black were produced, the parents must have been Ss.Bb and ss.Bb. The theoretical ratio would be 3 sooty (1 Ss.BB and 2 Ss.Bb), 3 type (1 ss.BB and 2 SS.Bb), and 2 black (1 sS.bb and 1 ss.bb). The expected ratio is 33 sooty, 33 type, and 22 black.

A sooty male paired with a black female produced 32 sooty, 16 sooty black, and 20 black. Since no type were produced, the male parent must have been homozygous for sooty, and since sooty black were produced, both parents must have carried sooty. The cross was therefore SS.Bb × sS.bb. The theoretical ratio would be 2 sooty (SS.Bb and Ss.Bb), 1 sooty black (SS.bb), and 1 black (sS.bb). The expected ratio is 34 sooty, 17 sooty black, and 17 black, to which the actual numbers although small are very close.

A pair, sooty male by sooty black female, and two pairs, sooty black males by sooty females, produced 66 sooty, 42 sooty black, and 16 black. Since black were produced, the sooty parents were Ss.Bb. The sooty black parents would of course be SS.bb.

This would give a theoretical ratio of 2 sooty (SS.Bb and Ss.Bb), 1 sooty black (SS.bb), and 1 black (sS.bb). The expected ratio is 62 sooty, 31 sooty black, and 31 black.

III. SUMMARY AND GENERAL DISCUSSION

Genetic work on the Mediterranean flour-moth has been handicapped by low fertility of the pairs, due for the most part to failure of females to oviposit.

Variations in size are apparently not hereditary.

Variations in length and divergence of leg spines, as well as abnormalities in external male genitalia, have not been tested genetically.

Defects in the labial palpi are hereditary but very irregular in appearance and probably much affected by environment.

The heredity of color variations and of cleft tongue will be discussed below.

A. The masking of a Mendelian difference by environment

It has for some time been recognized that the failure of a genetic character difference to appear is not necessarily due to the absence from the germ-plasm of the genetic factor or factors involved.

Baur ('11) has shown that in Primula sinensis both a genetic factor and certain environmental conditions are necessary for the production of pigment, and in Drosophila Morgan ('15) has demonstrated this for production of abnormal abdomen and Hoge ('15) for supernumerary legs.

In moths with cleft tongue, maxillae fail to unite for a variable part of their length. This abnormality apparently depends for its expression on certain environmental conditions, among which humidity is important. The hereditary basis may be a single factor difference from the normal, but further work will be necessary in order to demonstrate this conclusively.

B. Reversal of dominance

Reversal of dominance by sex has been called sex-limitation by Morgan ('14). This phenomenon has been demonstrated for horns in sheep by Wood ('05), for supernumerary mammae in swine ('12), and color in Ayrshire cattle ('16) by Wentworth, and for color in the butterfly, Colias philodice, by Gerould ('11).

Variations in dominance are frequent and are due to environment, age, or genetic constitution.

The present studies show a perfectly clear case of reversed dominance due to a simple Mendelian difference. Sooty is a simple dominant to type, while black is a simple recessive. In the homozygous black moths, however, sooty acts as a recessive. The dihybrid ratio, therefore, approximates 9 sooty, 3 type, 1 sooty black, and 3 black. Other ratios are discussed in the text, and it is suggested that reversal of this sort may be expressed by reversing the symbols denoting the factors. Thus heterozygous sooty is Ss.BB while heterozygous sooty black is sS.bb. A possible explanation for the reversal of dominance in this case is suggested under the discussion of the physiology of color production.

C. Analysis of a case of continuous variation

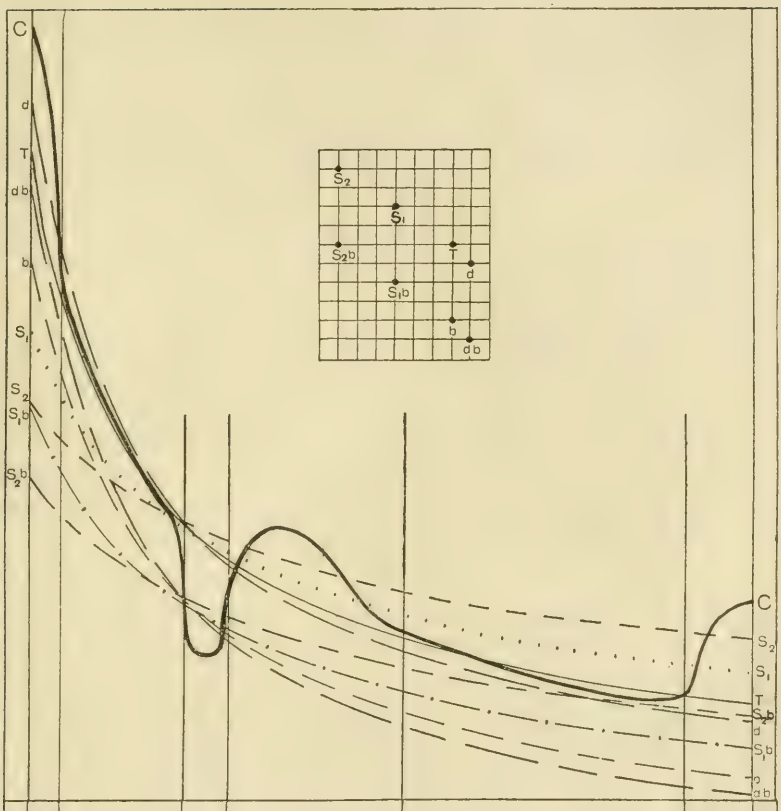
The separation of sooty base from type was at first impossible, as moths in single fraternities varied continuously from the most pronounced sooty to undoubted type. In later generations the segregation became sharp in some fraternities, but in others the intergradation persisted. A hereditary variation which darkened slightly the base of the wings, thus showing a slight tendency towards sooty, was probably responsible for some of the confusion. By far the greatest difficulty, however, resulted from the variation known as dark mid-area or dark. This variation has an effect somewhat the reverse of sooty base. It shows much intergradation with type, but probably depends upon a simple Mendelian difference. The nature of the reverse effect of sooty and dark is further discussed in the following section.

D. The physiology of color production

As regards coloration of the upper side of the fore-wing there is striking symmetry of the proximal to the distal half. In the type moth there are black marks near the center. The gray mid-area is bounded distally and proximally by outer and inner transverse, wavy, black lines. Next to these are the outer and inner narrow whitish lines and then the gray marginal and basal areas. At extreme margin and base are small black marks. In the color variations studied, symmetry is not lost, although certain of the markings mentioned are obliterated by intensification or dilution of pigment.

Color variations may be supposed to depend upon relative variations in quantity of a color producer and a color inhibitor. Figure 1 expresses the genetic and physiological relationships of color production in the proximal half of the fore-wing. The conditions in the distal half would be shown by a diagram symmetrical to this. Passing from left to right are shown as labeled the conditions at extreme base, basal area, inner white line, inner black line, inner half of mid-area, and inner half of median spots. The solid heavy curved line labeled *C* expresses the quantity of color producer present in the various regions of the wing. It is probable that this is not a single substance, but rather a color base and an activating enzyme. The color base may be present in greatest amount at extreme base of wing, black line, and median spots and in least amount in white line. The enzyme may decrease constantly in amount towards center of wing. The combination of the curves representing quantities of base and enzyme would approximate the curve *C*.

The other curves express relative quantity of inhibitor in each region for each of the color varieties studied. All of these curves are of exactly the same form and each is symmetrical with relation to a line drawn through the similarly labeled point in the ruled rectangle and at an angle of 45° to the horizontal. The curves would therefore coincide where the points superposed. The displacement of these points relative to each other represents, therefore, the displacement of the curves.



	Extreme base	Basal area	White line	Black line	Half of mid-area	Half of median spots
T	Black	Gray	White	Black	Gray	Black
b	"	Black	"	Black	Black	"
S_1 or S_2	"	Black	"	Black (restricted)	Very light gray	"
S_1b	"	Black (intense)	"	Black	Black (dilute)	"
S_2b	"	Black	"	Black	Very dark gray	"
D	"	Rather light gray	"	Black (extended)	Very dark gray	"
Db	"	Black (dilute)	"	Black	Black (intense)	"

Fig. 1

Neither the forms of the curves nor the displacements of the points are to be taken as in any way exact. They are merely approximations which express the genetic and apparent physiological relationships involved.

Curve and point *T* show amount of inhibitor in the type moth. The colors of the regions of the wing are printed below in the line marked *T*. Dots, curves, and colors of regions are correspondingly labeled for the other color varieties. The regions of the wings are not, of course, marked off definitely as expressed by the perpendicular lines, but grade into each other more or less as shown by the curves.

The recessive variation, black, (b), is represented as a general lowering of the inhibitor. Its point is placed four squares directly below that for type and its curve is correspondingly an equal distance below the type curve. Black, therefore, inhibits pigment formation only in the white lines. Variation in this respect obtains among individuals of black cultures. The moth represented in figure *o* has only a trace of the white lines.

The variation, dark mid-area, (d), darkens mid-area and lightens base. Its point is placed one square below and to the right of type. Its curve then is somewhat below that for the color producer in the mid-area and slightly above it towards the base. Dark black has its point and curve four points below dark. It may be seen to bear the same relation to black that dark does to type. A comparison of the curves of black and dark black shows how the 'ghost pattern' of dark seen in dark black is formed. The greater distance from the color producer curve of the curve for dark black at mid-area and the lesser distance towards the base accounts for the greater intensity of the mid-area.

There is, as stated, much intergradation with type in the dark mid-area moths. The points and curves shown in the diagram represent only one grade.

Sooty base has an action somewhat reverse to dark mid-area. Heterozygous sooty (S_1) has its point two squares above and three squares to the left of type and homozygous sooty (S_2) has its point twice that distance from type. This brings the curves

above the developer in mid-area and below towards base. They cross the curve for color producer at almost the same point and are each at sufficient distance above or below to indicate considerable dilution or intensification of pigment. Hence there is almost complete dominance of sooty despite the fundamental physiological difference between homozygote and heterozygote as expressed by the distances between their points and between their curves.

This fundamental difference is revealed in the reversal of dominance produced by black. It will be noticed that the points for sooty black are four points below the corresponding points for sooty. Black heterozygous for sooty (S_1b) therefore has its curve entirely below the developer curve, except at the white line, while the curve for homozygous sooty black (S_2b) closely approaches the developer curve in the mid-area, thus producing dilution of pigment. The difference in the course of the curve for black heterozygous for sooty from the curve for black accounts for the 'ghost pattern,' which is as expected the reverse of that seen in dark black.

The explanation here suggested for the reversal of dominance shows that while black acts as a dominant over sooty black, it is really an 'absence,' a lack of something possessed by sooty black. Let it not be supposed that this has anything to do with the much discussed 'presence-absence' theory. We are here dealing with physiological phenomena which are correlated with genetic factors. The greater amount of inhibitor in gray moths corresponds with the presence of the factor for gray and the absence of the factor for black, while the lesser amount in black moths corresponds with the presence of the factor for black and the absence of the factor for gray.

Curves corresponding with sooty dark are not shown. The effect of dark upon sooty is obvious, since the points for the two move in opposite directions from type.

The hypothesis advanced here to explain color production might be modified so that certain of the factors should affect the concentration of the activating enzyme rather than that of the inhibitor. In this case the displacement of the enzyme

curves would of course be in the opposite direction from that assumed for the inhibitor curves.

While the curves for inhibitors have all been drawn for convenience to the same form, it is obvious that the facts are in agreement with the supposition that the curves represent waves of slightly different lengths. In general the crests are at base and outer margin, while the troughs are in line with the median spots. Dark shortens the wave, making the trough slightly deeper. Sooty lengthens the wave considerably, making the trough shallower. Black merely reduces quantitatively without changing the form.

Changes in length of waves may be compared to the effect of the different factors in the banding series in cats (Whiting, '18). Bands of three different widths as seen in tabby cats are determined by three factors apparently allelomorphic. The narrower bands are dominant over the wider. It seems probable that in this case the bands represent waves of varying metabolic activity rather than concentration of definite enzymes or inhibitors.

Change in quantity of inhibitor without change of form of waves may be compared to agouti series in mammals. Individual hairs show bands of yellow more or less wide according to the quantity of inhibitor for black present. It is probable that the length of waves of concentration is not modified.

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PLATE 1

EXPLANATION OF FIGURES

a. Oblique view of head showing median palpus and tongue with coil pushed to the side.

b Side view of tip of male abdomen.

c Side view of head of type moth, showing tongue partially uncoiled.

d Oblique view showing tongue uncoiled and palpi asymmetrical.

e Side view of tip of female abdomen.

f Side view of head of black moth, with normal mouth parts.

g Ventral view, palpi very small, tongue cleft to base with elements diverging laterally.

h Side view showing tongue uncoiled and partially cleft.

i Ventral view, pupal cap retained, tongue cleft to base and elements coiled at sides.

j Side view of head of black tongueless moth.

k Ventral view of head of black tongueless moth.

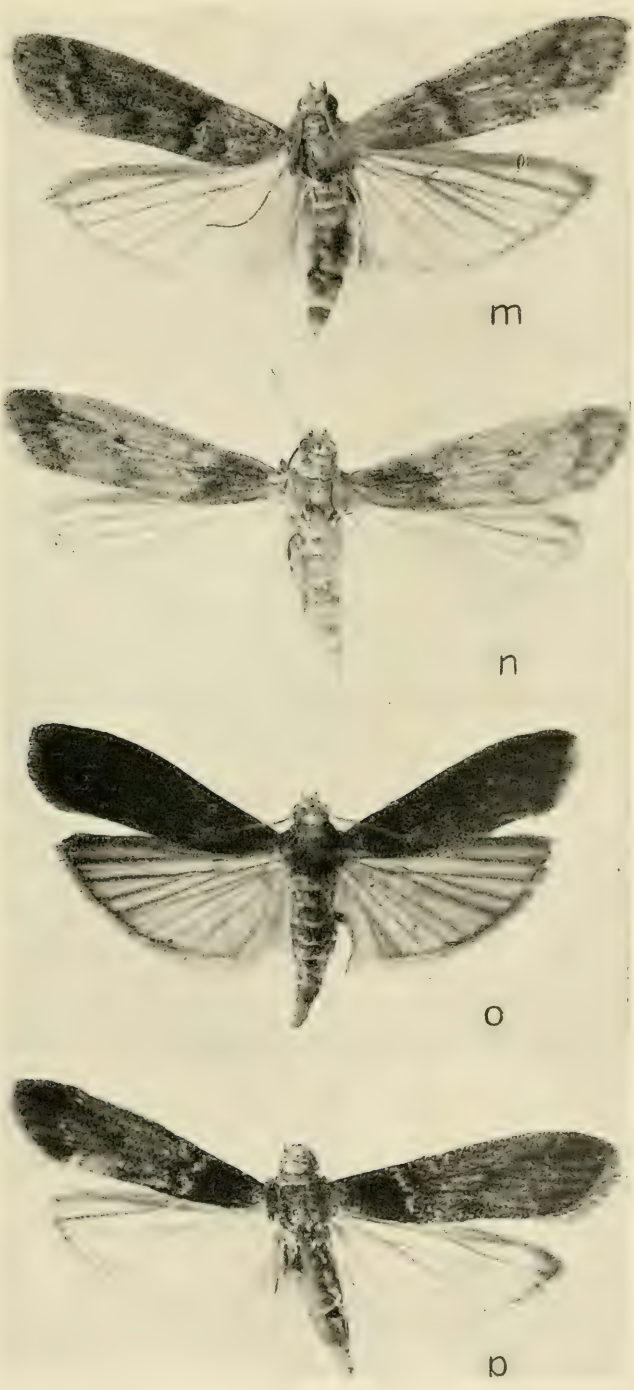
l Side view showing tongue uncoiled and palpi short.

a
b
c
d
e
f
g
h
i
j
k
l

PLATE 2

EXPLANATION OF FIGURES

m Type moth, ss. BB (b).
n Sooty-base moth, SS (s). BB (b).
o Black moth, ss (S). bb.
p Sooty black moth, SS. bb.


m
n
o
p

Resumen por el autor, W. P. Davey.
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Prolongación de la vida de Tribolium confusum debida aparentemente a pequeñas dosis de los rayos X.

En un trabajo publicado anteriormente (Journ. Exp. Zool., t. 22, múm. 3, Abril 1917) se describieron experimentos que demuestran: 1) Que una cantidad suficiente de rayos X pueden acortar la vida de Tribolium confusum, y, 2) Que la duración de la vida después de la acción de dichos rayos puede expresarse por una fórmula matemática, cuya derivación teórica fué expuesta en dicho trabajo. El presente trabajo contiene los resultados de nuevos experimentos, los cuales demuestran: 1) Que es posible, aparentemente, el prolongar de un modo material la vida de Tribolium confusum susministrándole pequeñas dosis de rayos X. 2) Que la prolongación de la vida, cuando se administran diariamente pequeñas dosis, es mayor que en el caso de administrarse dosis mayores de una sola vez. 3) El efecto letal de una dosis de rayos X es menor si se divide en una serie de dosis pequeñas administradas diariamente que cuando se somete al organismo a la acción de una sola dosis mayor. 4) El autor describe un método de análisis gráfico de los resultados obtenidos, mediante el cual el número de causas de muerte puede determinarse por la cantidad de individuos muertos y también estudiarse el efecto de un agente externo sobre cada una de estas causas. 5) Habiéndose empleado la misma clase de organismo durante todo el experimento los resultados comunicados en el presente trabajo y en el publicado anteriormente demuestran que por una simple variación de la dosis, un agente puramente físico (rayos X), puede producir a voluntad: a) una estimulación; b) un efecto destructivo que tiene lugar solamente después de un periodo latente y c) un efecto destructivo instantáneo.

Translation by José F. Nonidez
Columbia University

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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PROLONGATION OF LIFE OF TRIBOLIUM CONFUSUM APPARENTLY DUE TO SMALL DOSES OF X-RAYS

WHEELER P. DAVEY

FOUR FIGURES

In a previous article[1] experiments were described which showed, 1) that x-rays, when given in sufficient quantity, were able to shorten the life of Tribolium confusum, and 2) that the length of life after x-raying could be expressed by a mathematical formula, the theoretical derivation of which was given. It is the purpose of this article to give the results of further experiments showing that it is apparently possible to lengthen materially the life of Tribolium confusum by giving sufficiently small doses of x-rays.

In the article quoted above curves were given showing that the minimum dose necessary to kill all the beetles was 500 $\frac{MAM}{25^2}$ at 50 KV.[2] Some of the less resistant beetles could be killed by smaller doses, but the curves for 100 and 200 $\frac{MAM}{25^2}$ at 50 KV. had portions in which the death rate was lower than that of the controls. Comment on this was reserved until it could be confirmed by further experiments. Ample confirmation has now been obtained.

The experiments undertaken fall into two groups: A, those in which very small doses of x-rays were given daily throughout the life of the beetles; B, those in which the x-ray dose was given all at one time, as in the work previously published. In each of

[1] Journal of Experimental Zoölogy, vol. 22, no. 3, April, 1917.

[2] I.e., 500 milliampere-minutes at 25 cm. distance at 50 'root-mean square' kilovolts.

these groups of experiments it has been shown possible to duplicate results time after time, subject only to those general limitations which are inseparable from biological work. Typical experiments in each group will be described below. Apparatus and technique were the same as in the work previously reported.

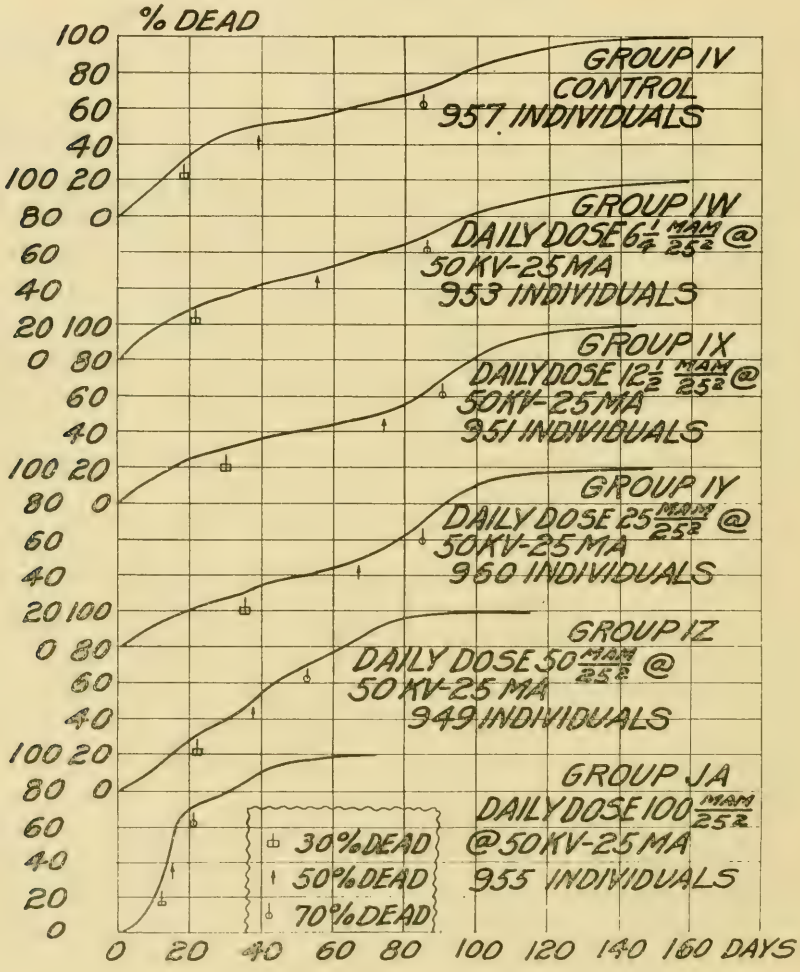

Fig. 1

A. PROLONGATION OF LIFE DUE TO SMALL DAILY DOSES OF X-RAYS

Six groups of approximately 950 individuals each were taken. These were known as groups IV, IW, IX, IY, IZ, JA.

Group IV was the control.

Group IW was given $6\frac{1}{4}\frac{MAM}{25^2}$ at 50 KV. – 25 MA. daily.

Group IX was given $12\frac{1}{2}\frac{MAM}{25^2}$ at 50 KV. – 25 MA. daily.

Group IY was given $25\frac{MAM}{25^2}$ at 50 KV. – 25 MA. daily.

Group IZ was given $50\frac{MAM}{25^2}$ at 50 KV. – 25 MA. daily.

Group JA was given $100\frac{MAM}{25^2}$ at 50 KV. – 25 MA. daily.

After 159 days the beetles were practically all dead. The data on the death rates were then collected and plotted as shown in figure 1. These graphs furnish ample proof that it is possible to reduce the death rate of Tribolium confusum by small daily doses of x-rays.

Table 1 gives readings from these graphs to the nearest whole number.

TABLE 1

NUMBER DAYS AFTER RAYING	PER CENT OF DEAD					
	Group IV, control	Group IW, $6\frac{1}{4}\frac{MAM}{25^2}$ at 50 kv. daily	Group IX, $12\frac{1}{2}\frac{MAM}{25^2}$ at 50 kv. daily	Group IV, $25\frac{MAM}{25^2}$ at 50 kv. daily	Group IZ, $50\frac{MAM}{25^2}$ at 50 kv. daily	Group JA, $50\frac{MAM}{25^2}$ at 50 kv. daily
10	17	17	14	11	12	20
20	34	29	25	21	28	69
30	46	35	30	28	39	79
40	51	42	36	34	55	90
50	54	47	40	39	67	96
60	58	53	44	44	77	99
70	63	59	48	52	88	100
80	67	65	56	63	96	
90	74	74	69	79	98	
100	84	83	84	91	99	

These readings, taken from the smooth curves of the graphs, do not differ from the actual experimental data by more than 1 per cent.

Except while being x-rayed or counted, the beetles were kept in an incubator at 34 to 35°C. In order to make sure that the results were not affected by some possible 'temperature coefficient of life,'[3] the controls were taken out of the incubator while group JA was being rayed, and were kept out during the whole raying. Since group JA was rayed the longest time each day, this meant that the controls were cooled off for a longer time than groups IW, IX, IY, IZ. Therefore, if cooling off for a few minutes each day happened to tend to increase the length of life, then the controls were made to live longer than they otherwise would. The actual increase in length of life observed in groups IW, IX, and IY is, therefore, not due to any possible effect of temperature, but occurs in spite of it. After so many boxes of beetles in JA were dead that the time of raying group IZ was greater than the time of raying JA, the controls were kept out of the incubator while group IZ was being rayed.

Some data not given in the graphs may be of additional interest. Each group was divided into two subgroups of about the same number of individuals each. It was found that the idiosyncrasy was great enough so that the curves of the corresponding subgroups could not be exactly superimposed. However, it was found that this idiosyncrasy was always less than the changes in death rate caused by x-rays. By way of illustration, table 2 shows the percentage of beetles dead in each subgroup, *a*) on the day when 50 per cent of the controls were dead; (*b*) on the day when 50 per cent of the x-rayed group were dead. This table shows that the lowest death rate among the controls (group IV) was higher than the highest death rate among the beetles of groups IW, IX, IY.

It is interesting to note in this connection that the total dose received by these beetles was greatly in excess of that minimum dose which, when given all at once, would have caused premature death.

[3] Loeb and Northrup, Proc. Nat. Acad. Sci., Aug., 1916.

TABLE 2

GROUP	PER CENT OF TRIBOLIUM CONFUSUM DEAD			
	Approximately 50 per cent controls dead		Approximately 50 per cent x-rayed tribolium confusum dead	
	Subgroup (1)	Subgroup (2)	Subgroup (1)	Subgroup (2)
	39th day		56th day	
IV IW	47.7 41.6	54.2 42.1	52.8 48.1	60.0 52.3
	39th day		74th day	
IV IX	47.7 32.4	54.2 38.3	59.9 44.7	70.3 54.1
	39th day		67th day	
IV IY	47.7 31.7	54.2 36.7	58.6 48.5	68.2 50.2
	39th day		38th day	
IV IZ	47.7 52.9	54.2 54.5	46.8 50.1	53.4 52.4
	39th day		14th day	
IV JA	47.7 88.5	54.2 91.7	22.1 43.7	24.9 46.8

A further analysis of the data of groups IV to JA will be of interest. The curves shown in figure 1 were each replotted on probability paper[4] (fig. 2). It was found that each curve was composed of portions of three accurate probability curves, joined end to end. It is as though there were three causes of death, or perhaps three definite groups of ages. These three portions of the death-rate curve will be termed A, B, and C. Portion C represents those beetles which lived the longest in their group.

[4] The ordinates of probability paper are so spaced that the ordinary curve of the probability integral is represented by a straight line.

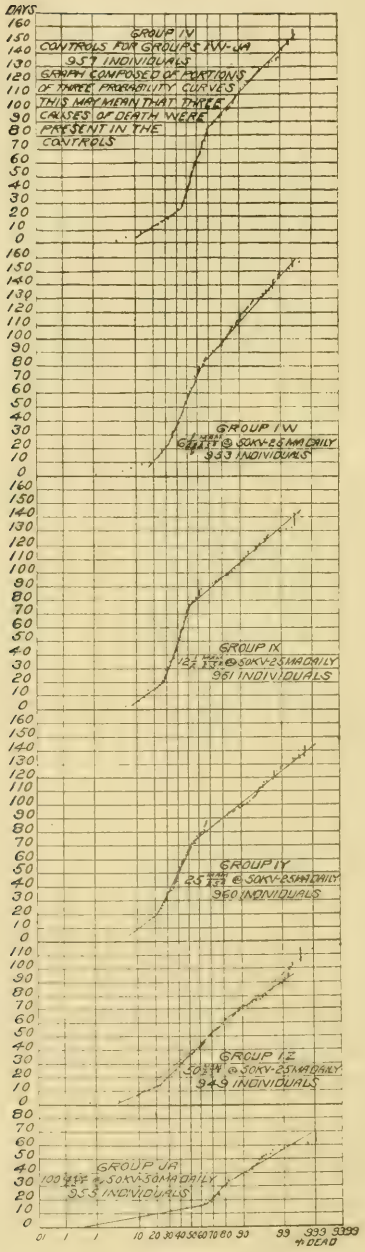
DAYS
GROUP IV
CONTROLS FOR GROUPS XV-JA
957 INDIVIDUALS
GRAPH COMPOSED OF PORTIONS
OF THREE PROBABILITY CURVES
THIS MAY MEAN THAT THREE
CAUSES OF DEATH WERE
PRESENT IN THE
CONTROLS
GROUP IW
933 INDIVIDUALS
GROUP IX
961 INDIVIDUALS
GROUP IY
960 INDIVIDUALS
GROUP IZ
949 INDIVIDUALS
GROUP JA
955 INDIVIDUALS
+DEAD

Fig. 2

TABLE 3

GROUP	DAILY DOSE	PER CENT DIED		
		A	B	C
IV	Control	44	26	30
IW	$6\frac{1}{4}$	32	36	32
IX	$12\frac{1}{2}$	26	26	48
IY	25	21	35	44
IZ	50	23	61	16
JA	100	64	17	19

Table 3 gives the death rate per 100 in each group for A, B and C.

It is evident that the smallest daily dose (group IW) decreases the death rate of 'A' and that those beetles which are kept from dying of 'A', die of 'B.' Deaths from cause 'C' are practically unaltered. A larger daily dose (group IX) causes about half of those which would normally die of 'A' to die of 'C.' A still larger daily dose (group IY) causes half of those which would have died of 'A' to die of 'B' and 'C.' A still larger daily dose (group IZ) acts much like the previous dose in causing almost half of those which would have died of 'A' to die of 'B,' but it differs from it in that some of those which would have died of 'C' are prematurely killed. The largest daily dose employed (group JA) caused about a third of those which would have died of 'B' and 'C' to die of 'A.'

It is hard to interpret all this. It may be that life cannot exist except in the presence of a small amount of radio-activity. The radio-activity of the earth may not have been of the optimum value, so that some benefit was derived from the x-rays received each day. The following is an effort at an alternative explanation. The evidence given by group JA shows that the lethal action of x-rays is tied up in some way with cause of death 'A.' It is well known that the lethal action of x-rays is more marked on cells in the process of division than on those in the resting state. Therefore, small daily doses (larger than a certain minimal value) can kill off those few cells which happen to be in a state of division at the time of raying. The death of

these few cells stimulates the production of more to take their places between the periods of raying. Therefore, small daily doses, instead of increasing the death rate from cause 'A,' actually decrease it by stimulating the processes of repair. The whole individual beetle, therefore, has a smaller chance of dying from 'A' and is compelled to die of either 'B' or 'C.' When the daily dose is increased to such a value that the daily destruction of cells is equal to or greater than the production of new cells, premature death occurs, from causes 'B' or 'A' (see groups IZ and JA).

B. PROLONGATION OF LIFE DUE TO SMALL SINGLE DOSES OF X-RAYS

Five groups of approximately 850 individuals each were taken. These were known as groups JB, JC, JD, JE and JF.

Group JB was the control.

Group JC was given $100 \frac{\text{MAM}}{25^2}$ at 50 KV. – 50 M.A.

Group JD was given $200 \frac{\text{MAM}}{25^2}$ at 50 KV. – 50 M.A.

Group JE was given $300 \frac{\text{MAM}}{25^2}$ at 50 KV. – 50 M.A.

Group JF was given $400 \frac{\text{MAM}}{25^2}$ at 50 KV. – 50 M.A.

The beetles were rather old, so that the controls were all dead on the fortieth day of the experiment. There were so few beetles still alive after the thirty-fifth day that the results of the last five days are not of the same order of accuracy as those of the first thirty-five days.

The first ten days of the experiment, group JC ($100 \frac{\text{MAM}}{25^2}$ at 50 KV.) had the same death rate as the controls. After the tenth day the death rate was considerably less than that of the controls. The two groups were divided into two equal subgroups, and although it was found that the idiosyncrasy was such that the subgroups were not exactly alike, still, after the

tenth day, the highest death rate of JC was lower than the lowest death rate of the controls.

During the first seventeen days of the experiment, group JD ($200 \frac{MAM}{25^2}$ at 50 KV.) had a higher death rate than the controls. After the seventeenth day the death rate of group JD was less than that of the controls. After the twentieth day the death rate of JD was identical with that of JC. When divided into two equal subgroups, as described above, it was found that after the twenty-second day the highest death rate of group JD was less than the lowest death rate of the controls.

During the first twenty-nine days of the experiment the death rate of group JE ($300 \frac{MAM}{25^2}$ at 50 KV.) was greater than that of the controls. After the twenty-ninth day the death rate of JE was less than that of the controls.

The death rate of group JF ($400 \frac{MAM}{25^2}$ at 50 KV.) was at all times greater than that of the controls.

These results are shown graphically in figure 3. Figure 4 contains an analysis of these same curves by means of probability paper, showing that, as in the case of experiment A, the curves are composed of accurate portions of probability curves placed end to end.

All of the above results seem to be a direct confirmation of the curves given in the previous paper (loc. cit.). The effect of concentrated single doses is not nearly so marked as the effect of a series of small 'homeopathic' doses. This seems to be much the same law as is already well known in serum therapy and in the action of certain drugs. In the case of serum therapy, this law has been shown to be identical with the law of adsorption. If it could be rigorously shown that the effects of exposure to x-rays follow the same general law, we should conclude that the x-rays are responsible for the production of some substance, perhaps in the blood, which is later adsorbed.

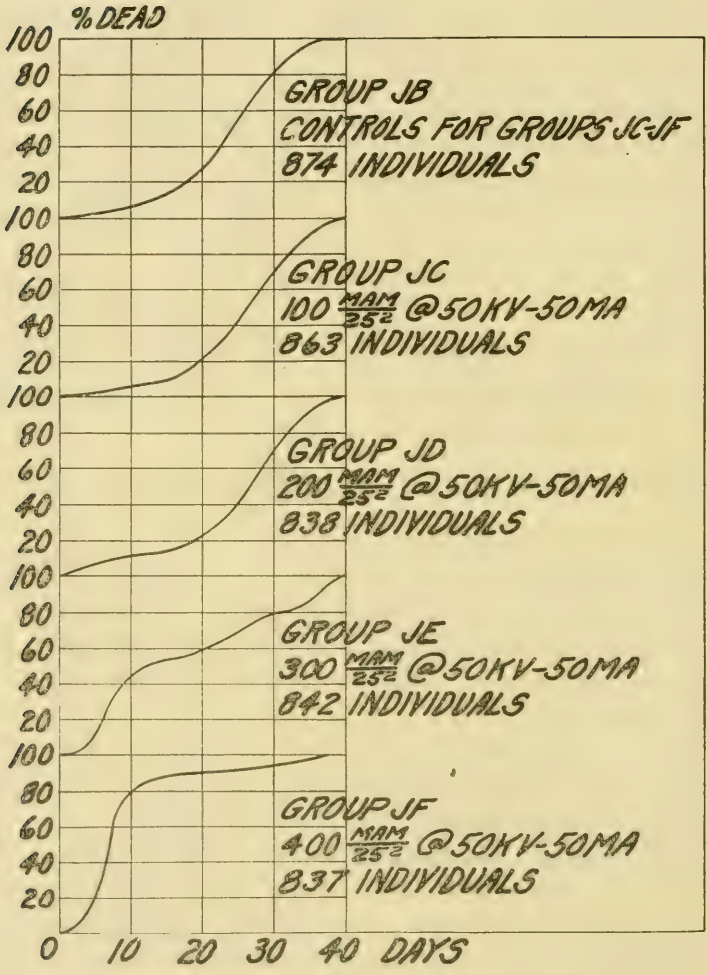
% DEAD
100
80
60
40
20
0
10
20
30
40
DAYS
GROUP JB
CONTROLS FOR GROUPS JC-JF
874 INDIVIDUALS
GROUP JC
100 MAM/25² @ 50KV-50MA
863 INDIVIDUALS
GROUP JD
200 MAM/25² @ 50KV-50MA
838 INDIVIDUALS
GROUP JE
300 MAM/25² @ 50KV-50MA
842 INDIVIDUALS
GROUP JF
400 MAM/25² @ 50KV-50MA
837 INDIVIDUALS

Fig. 3

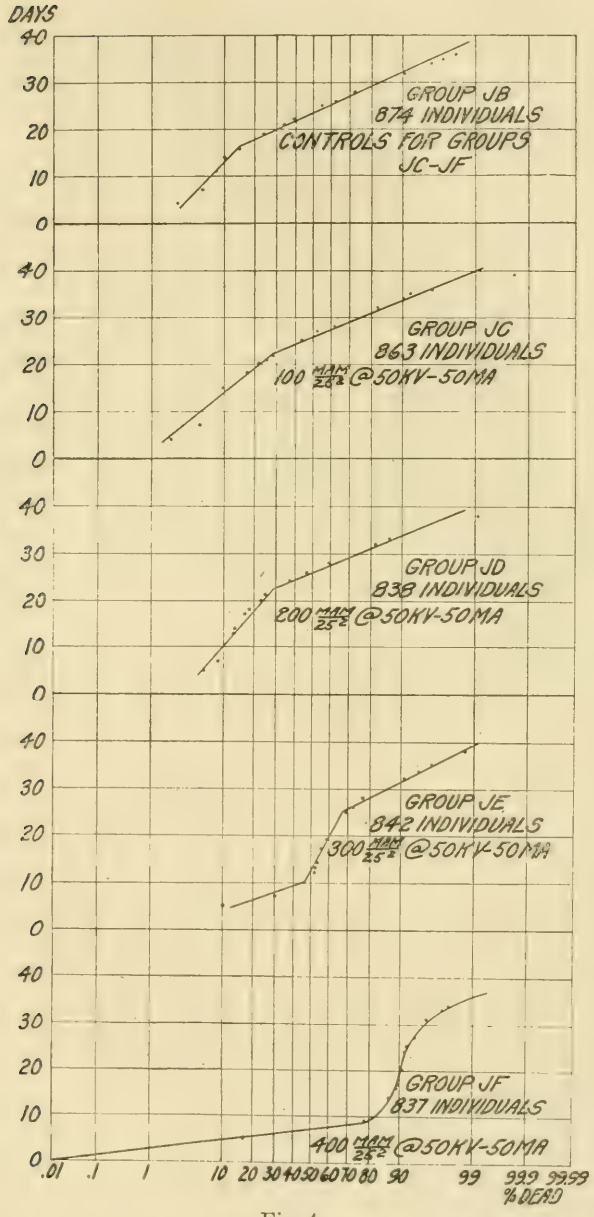
DAYS
GROUP JB
874 INDIVIDUALS
CONTROLS FOR GROUPS
JC-JF
GROUP JC
863 INDIVIDUALS
100 MAM/25² @ 50KV-50MA
GROUP JD
838 INDIVIDUALS
200 MAM/25² @ 50KV-50MA
GROUP JE
842 INDIVIDUALS
300 MAM/25² @ 50KV-50MA
GROUP JF
837 INDIVIDUALS
400 MAM/25² @ 50KV-50MA
.01 .1 1 10 20 30 40 50 60 70 80 90 99 99.9 99.99
% DEAD

Fig. 4

SUMMARY

1. It has been shown that the life of Tribolium confusum may be prolonged by the use of a purely physical agent, i.e., x-rays.

2. The prolongation of life due to a series of small daily doses is greater than that of larger doses given all at once.

3. The lethal effect of an x-ray dose is less if it is split up into a series of small daily doses than if it is given all at once.

4. A method of graphical analysis of results has been described by which the number of causes of death may be determined from the death rate, and by which the effect of an external agent upon each of these causes may be studied.

5. Using the same kind of organism throughout the whole experiment, the work reported in this and the previous paper has shown that, by merely varying the size of the dose, a purely physical agent (x-ray) may be made to produce at will, 1) a stimulation; 2) a destructive effect which occurs only after a latent interval, and 3) an instant destructive effect.

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Resumen por el autor, Carl R. Moore.

Sobre las propiedades de las gonadas como reguladores de los caracteres somáticos y psíquicos

II. Crecimiento de las ratas machos y hembras gonadectomizados

El autor da una descripción del crecimiento de ratas después de separar las glándulas sexuales en los individuos jóvenes de ambos sexos. Estos experimentos se emprendieron para determinar si existe una diferencia constante en el peso de los individuos de ambos sexos (como sucede en el crecimiento normal) aparte de una influencia secundaria de las glándulas sexuales. Los resultados obtenidos demuestran de una manera definitiva que hay una diferencia potencial de peso en los dos sexos, la cual no está relacionada con la influencia que ejercen las glándulas sexuales; los machos pesan invariablemente mas que las hembras.

Translation by José F. Nonidez
Columbia University

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JUNE 2

ON THE PHYSIOLOGICAL PROPERTIES OF THE GONADS AS CONTROLLERS OF SOMATIC AND PSYCHICAL CHARACTERISTICS

II. GROWTH OF GONADECTOMIZED MALE AND FEMALE RATS

CARL R. MOORE

Hull Zoological Laboratories, The University of Chicago

ONE FIGURE

During a study of the effects of transplanted gonads in modifying somatic and psychical characteristics in mammals,[1] it was highly desirable to know the relations of the growth curve of gonadectomized male and female rats before the effects of homoplastic transplantation of gonads could be correctly interpreted.

Many investigators have shown that the growth curve of normal male rats is consistently and considerably above that of normal female rats.[2] Steinach,[3] after gonadectomy of young male and female rats and subsequent transplantation to each of the gonad of the opposite sex, noticed that the growth (weight) of the females had a tendency to be above the normal for females and that of the males to be lowered from the normal for unoperated males, provided the transplantations were successful; and he has not only associated these changes with a supposed modification of sex, but has used the changes as a criterion of sexual changes.

Stotsenburg, however, in a careful series of experiments, has shown that the elimination of the ovaries of young female rats, without the subsequent transplantation of testis, causes the growth curve of the spayed females to be increased 17 per cent

[1] Moore ('19).
[2] See Donaldson ('15).
[3] See Steinach ('10, '11, '12, '13).

to 30 per cent above that of normal females;[4] he has proved also that the removal of the testis from young males does not influence the subsequent growth curve as compared with normal males.

As the presence of the gonads, at least in case of the female rat, is a modifying element of the growth curve, it is highly desirable to know whether there is a difference in the growth of the two sexes aside from any influence exerted by the gonads. Is there a latent, potential, sex difference between the male and female growth aside from any modification brought about by the presence or absence of the sex glands? Stotsenburg's data as presented are not adequate for giving an answer to this question.

This paper contains the results of a comparison of the growth of completely castrated males and spayed females of the same litters and shows conclusively that there is constantly a difference in weight in the two gonadectomized sexes. The spayed females do increase in weight relative to the normal females and approach more nearly the weight of the males, but in every case the males are heavier than the females.

MATERIAL

Seven litters of the common white rat (Mus norvegicus albinus), composed of fifty-four rats, forty of which were castrated or spayed, the remaining fourteen serving as controls, constitutes the material used. Each litter was kept in a separate cage, but in the same room during their growth, and the entire experiment was confined to the period from November 29, 1917, the birth of the oldest litter used, to September 1, 1918, when the last weighing was made; and since the weight of each litter was recorded for 180 days, it will be realized that corresponding weights for each litter were made at comparatively the same time of year.

The diet used was a constant one and consisted of milk and bread daily, a small amount of meat twice each week mixed

[4] Stotsenburg ('13).

with the bread and milk and a small amount of grain (corn or oats) placed in the cage once each week. The rats were allowed to eat all they would at one time each day and weighings were made seven to ten hours after the meal.

Gonadectomy was performed under anesthesia at ages of twenty-three to thirty days, and the gonads were removed from both sexes by a midventral incision.

Table 1 is a tabulation of the material used; the normal male and female served as controls, though the relation of operated to unoperated animals is not considered in this paper.

TABLE 1

LITTER	AGE AT OPERATION	NUMBER OF MALES CASTRATED	NUMBER OF FEMALES SPAYED	CONTROLS NORMAL
	days			
11 A^3B^3	23	2	6	1♂, 1♀
30 A^1B^1	26	3	3	1♂, 1♀
48 A^1B^1	30	3	3	1♂, 1♀
24 A^1B^1	51	2	2	1♂, 1♀
17 A^1B^1	29	3	3	1♂, 1♀
25 A^2B^2	26	3	3	1♂, 1♀
38 A^1B^1	31	2	2	1♂, 1♀
Totals seven litters		18	22	

Stotsenburg has already stated that, at about 200 days old, many times rats are affected by certain ailments that interrupt the ascent of the normal growth curve, and for this reason, as well as on account of the death of several animals, the weight records only include growth up to 180 days. But considerably before this time the rats would have been sexually mature and entirely adult.

Table 2 is a tabulated record of the average weight of individuals of each sex in each litter from the time of operation up to the 180th day, as well as the number of individuals of each sex in the litter at that age; the last column is the percentage of the weight of the males above that of the females at each weighing, the percentages having been calculated from the average weight of the individuals of each sex as given in the table. An

TABLE 2

AGE IN DAYS	CASTRATED MALES		SPAYED FEMALES		WEIGHT OF MALES ABOVE FEMALES
	Number of animals	Average weight	Number of animals	Average weight	
			Litter 11 A^3B^3		
days		*grams*		*grams*	*per cent*
23	2	25.0	6	24.6	1.6
46	2	58.0	6	53.3	9.7
59	2	91.0	6	79.5	14.4
90	1	122.0	6	106.6	15.0
120	1	170.0	6	140.1	21.4
150	1	208.0	6	165.0	26.0
180	1	207.0	6	162.4	27.0
			Litter 30 A^1B^1		
26	3	26.0	3	26.3	−1.1
46	3	58.0	3	56.0	3.5
59	3	93.6	3	85.0	10.1
90	3	124.0	3	109.6	13.1
120	3	167.0	3	142.0	17.6
150	3	199.0	3	174.0	14.3
180	3	188.3	3	164.0	14.8
			Litter 48 A^1B^1		
30	3	22.0	3	19.6	12.2
43	3	30.6	3	26.6	15.0
60	3	59.0	3	50.0	18.0
90	3	110.6	3	93.6	18.1
120	3	136.0	3	122.6	10.9
150	3	142.6	3	130.0	9.6
180	3	161.6	3	148.0	9.1
			Litter 24 A^1B^1		
51	2	55.0	2	49.0	12.2
97	2	128.0	2	117.5	8.8
120	2	152.5	2	136.0	12.1
150	2	178.0	2	169.0	5.3
180	2	185.0	2	172.5	7.2
			Litter 17 A^1B^1		
29	3	27.0	3	27.0	
45	2	48.5	3	49.6	−2.2
60	2	73.0	3	74.3	−1.3
90	2	106.0	3	100.0	6.0
120	2	149.5	3	134.6	11.0
150	2	163.5	3	139.3	17.3
180	2	176.5	3	155.0	13.2

TABLE 2—*Continued*

AGE IN DAYS	CASTRATED MALES		SPAYED FEMALES		WEIGHT OF MALES ABOVE FEMALES
	Number of animals	Average weight	Number of animals	Average weight	
Litter 25 A^2B^2					
days		*grams*		*grams*	*per cent*
26	3	28.6	3	27.3	4.7
41	3	54.6	2	47.0	16.0
63	3	88.6	2	78.5	12.8
90	3	130.3	2	95.5	36.4
120	3	157.3	2	125.0	25.8
150	3	190.3	2	148.5	28.1
180	3	195.0	2	156.5	24.6
Litter 38, A^1B^1					
31	2	35.0	2	31.0	12.9
44	2	59.0	2	51.0	15.6
60	2	89.0	2	85.5	4.0
90	2	117.5	2	111.5	5.3
120	2	161.5	2	148.5	8.7
150	2	194.5	2	177.0	9.0
180	2	199.0	2	182.5	9.0

examination of this percentage column shows that only in four instances was the average female weight equal to or greater than the average male weight of a given litter. The weight of the males in these four cases, not only reached that of the females before the age of sixty days, but increased gradually as growth continued.

By using the average weight of each sex of each litter at comparative ages as given in table 2, the average weight of the males and females of all seven litters has been expressed in the form of a growth curve in figure 1. The broken lines from thirty to sixty days is only approximately correct due to the fact that all litters were not weighed at exactly the same age prior to the sixtieth day.

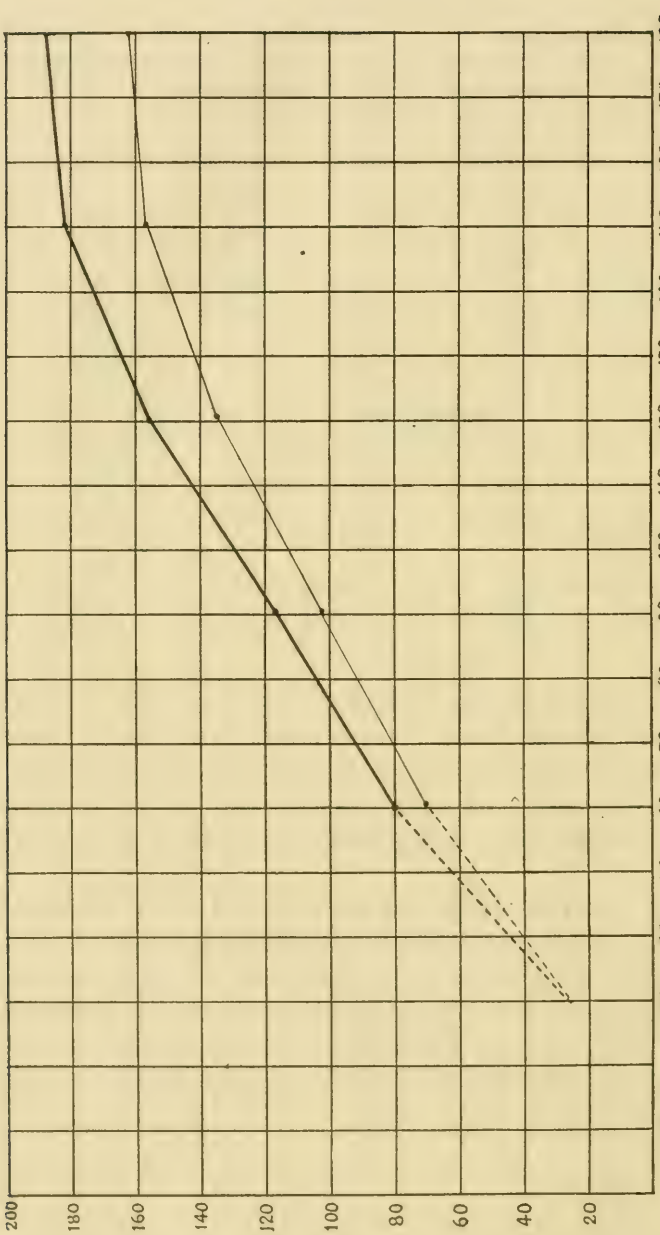

Fig. 1 Curve of growth of gonadectomized male and female rats. The ordinates represent grams of body weight and the abscissae the age of the animals in days. The upper curve represents the growth of castrated males, the lower one of spayed females. The curves were constructed by using the average weight of each sex of each litter at the designated age in days (table 2); from this the average weight of each sex for all litters at given ages were computed and are represented by the curves. The broken part of the curves from thirty to sixty days is due to the fact that, at the beginning, all litters were not weighed at exactly the same age; this part of the curve is only approximately correct.

DISCUSSION

The primary object of these experiments was, first, to determine to what degree constant weight differences between the normal male and female rat were determined by the sex glands, and, second, to provide a basis for interpretation of weight differences in case of homoplastic transplantation of the gonads.

In studying the effects of the influence of the gonad of one sex on modifying the somatic and psychical development of the opposite sex (a repetition of the experiments of E. Steinach), the writer has differed from Steinach in the interpretation of the results obtained. Steinach has placed considerable emphasis on modification of body weight of rats and guinea-pigs following removal of the normal gonads and the substitution of the opposite one by transplantation. If these transplantations were successful—i.e., if the graft persisted and grew—according to Steinach, the male became 'feminized' and, compared with normal males, relatively decreased in weight as development proceeds, while the female became 'masculinized' and correspondingly increased in weight in comparison with unoperated females. These changes from the normal weight for the sex he associates with the presence of the secretion of the implanted gland; the female increased in weight because a secretion from the testis was present in the female into which it had been placed, and having this male secretion the weight of the individual increases toward the normal weight of a male and away from that of a female. In case of a secretion of the ovary in a male animal, the weight of this feminized male approaches that of a normal female.

Stotsenburg ('09), however, has shown for rats, that the presence of the secretion of the testicle has absolutely no influence upon the growth of the individual. Also ('13) he has proved that the mere removal of the ovaries of young rats results in an increase of from 17 per cent to 33 per cent compared with unoperated females.

Considering these findings, the writer has found it impossible to associate weight differences with different degrees of maleness

or femaleness in all cases after transplantation of the gonad of the opposite sex. The female increases in weight not because of the influence of the secretion from the transplanted testis, but solely on account of the removal of the ovary, which alone seems to have any influence upon the growth of the animal. There seems to be no doubt that the presence of the ovary does prevent the normal ascent of the growth curve. In order to know whether this sex difference in weight was due entirely or only in part to the influence of the secretions of the gonads, the preceding experiment was carried out. The results show very conclusively that there is a real difference between the capacity of the two sexes to accumulate somatic materials when there are no secondary influences that may be attributed to the influence of the gonad. As table 2 shows, this difference has been exhibited at each stage by each of the seven litters used.

It is interesting to consider this potential weight difference of the 'determined male' and the 'determined female' in their development in the light of Riddle's theory of sex.[5] Riddle and his co-workers have demonstrated actual differences in the chemical constitution of male-producing and female-producing eggs of the pigeon. He has not only shown that the female-producing egg contains a greater phosphatide content and a lesser percentage of water, but he has also demonstrated that these chemical differences found in the dimorphic ova of birds are carried over into the adult life of the bird.[6] His idea is that sex determination is based upon a higher rate of metabolism of the ovum producing a male than of that producing a female, and several researches are cited to show that the same relative rate of metabolism persists in the adult male and female. It has occurred to the writer that these basic differences in weight of the two sexes of rats may also indicate a possible difference in metabolism inherited from the original ovum from which each had been developed. But aside from the primary differences that may exist in the determined male or female, the secondary influences that make the female a more apparent

[5] Riddle ('17).

[6] Lawrence and Riddle ('16).

female and the male a more apparent male are due to the presence of the specific gonads, and these differences, in many cases, have proved themselves capable of being controlled to a certain extent.

CONCLUSIONS

After early removal of sex glands the growth curve of the determined male is (without exception in these experiments) higher than that of the determined female. There is, therefore, a real difference (of metabolism?) in the two sexes, which may represent an inherited difference from the original ova, but this difference may be accentuated by the presence of the ovary in the female.

Hull Zoological Laboratories,
The University of Chicago,
February 17, 1919.

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Resumen por el autor, D. D. Whitney.
Universidad de Nebraska.

La inefectividad del oxígeno como factor causante de la producción de machos en Hydatina senta.

Los cultivos del flagelado microscópico Chlamydomonas desprenden cantidades considerables de oxígeno libre cuando se colocan a la luz solar, hasta tal punto que el agua del cultivo puede contener hasta + 16 cc. de oxígeno libre por litro, mientras que en la oscuridad no hay desprendimiento de dicho gas. El autor colocó los rotíferos en dichos cultivos, a la luz solar y en la oscuridad, sirviéndoles de alimento los Chlamydomonas. Los primeros, aun a pesar de estar en un medio rico en oxígeno libre, originaron menos hijas productoras de machos que los colocados en la oscuridad, en los cuales la cantidad de oxígeno libre es mucho menor. Este resultado se debe probablemente a la alimentación. En la luz solar los individuos de Chlamydomonas se reunen en la superficie del agua y en las paredes de la vasija de vidrio que los contiene y de este modo se hacen inacesibles como alimento de los rotíferos. Por el contrario en la oscuridad permanecen nadando activamente en el agua del cultivo durante 3 a 4 dias y los rotíferos pueden comerlos fácilmente. La cantidad de hembras productoras de machos resultó ser la misma en el agua de cultivo con una cantidad mínima de oxigeno libre (1 a 3 cc. por litro) y en la que contenía una cantidad mayor de este gas (2 a 8 cc. por litro).

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THE INEFFECTIVENESS OF OXYGEN AS A FACTOR IN CAUSING MALE PRODUCTION IN HYDATINA SENTA[1]

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Recent papers by Shull and Ladoff ('16) and Shull ('18) maintain that a sufficient amount of dissolved oxygen in the culture water will cause an increase in the production of males in the rotifer Hydatina senta. Whitney ('17) explained these results obtained by Shull and Ladoff as being due to the effect of oxygen upon the food supply which in turn effected the production of males. Later, Shull made further experiments which seem to demonstrate that oxygen is really a potent influence in causing males to be produced. Not being convinced, however, that the problem was finally settled, new experiments were undertaken by the author, which in their turn seem to show that oxygen in itself is not effective in causing males to be produced.

METHOD

In all of the experiments old culture water was used which had been made out of rain water and horse manure several weeks or months previous to the beginning of the present experiments. This culture water was such that when it was a few weeks old rotifers readily lived in it, and they also lived in it just as readily when it was several months old provided food was put into it.

The best food supply in these experiments seemed to be a mixture of green flagellates, Chlamydomonas, and colorless flagellates, Polytoma. A pure diet of Chlamydomonas used for several successive days was detrimental to the rotifers. Many of them would gorge themselves on this food so that their stomachs would burst and allow the contents to fill the whole body

[1] Studies from the Zoological Laboratory, The University of Nebraska, no. 121.

cavity, thus causing death, while in others the Chlamydomonas would form a dense and compact mass in their stomachs which also caused death. By mixing a small proportion of Polytoma with the Chlamydomonas, both of these troubles were avoided. The Chlamydomonas was raised in large quantities in bouillon solution in direct sunlight and the Polytoma was raised in stable tea in darkness. The details of rearing both of these flagellates have been published in former papers.

In many of the experiments the amount of food was measured in a graduated pipette. The Chlamydomonas and the Polytoma were each separately centrifuged and all of the original culture water drained off. Then just enough old stable-tea culture water was added to allow the Chalmydomonas and Polytoma to be drawn up into the pipette. In this way the amount of food could be quite accurately measured and regulated at will in each experiment.

It was found that the Chlamydomonas could be transferred from the sunlight to absolute darkness and would remain alive and active for several days, the duration of activity being somewhat dependent upon temperature.

The amount of oxygen, number of cubic centimeters per liter, whenever determined in the experiments was determined by the Winkler method described in Standard Methods of Water Analysis published by the American Public Health Association of Boston. The sodium thiosulphate solution was standardized against potassium dichromate about three times per week.

The following experiments are not arranged chronologically, but are so arranged as to present the evidence and data in a logical manner.

EXPERIMENTS SHOWING THE PRODUCTION OF OXYGEN BY CHLAMYDOMONAS IN DIRECT SUNLIGHT

It was considered desirable to determine how much free oxygen is given off by Chlamydomonas when the culture is in the direct sunlight for several hours. Varying quantities of Chlamydomonas were put into about 50 cc. of old stable-tea culture water and poured into stender dishes, 1 inch in diameter,

and placed in a pan of running water in the direct sunlight. The running water maintained a temperature of 20° to 25°C. The old stable-tea culture water had been standing in a north light for several months and had only a small amount of free oxygen in it. This made an excellent starting solution. Usually after a short time in the sunlight the Chlamydomonas would emit free oxygen in sufficient quantities to rise to the surface in minute bubbles. After several hours in the sunlight there was formed usually a frothy scum on the surface which was composed of these minute bubbles of oxygen.

Table 1 shows some of the details and the results of these few experiments. Experiments 1 and 3 show the amount of oxygen generated in periods of one hour, two hours, and four hours,

TABLE 1

Showing that in sunlight the green flagellates, Chlamydomonas, give off considerable quantities of free oxygen in old stable culture water that is devoid of all food substances

EXPERIMENTS	LOTS	LIGHT CONDITIONS	TIME, 1918	CULTURE WATER	CHLAMYDOMONAS	CULTURE WATER TESTED	OXYGEN PER LITER
				cc.	*cc.*	*cc.*	*cc.*
1	A	North light	Several weeks	42	0	42	5.31
	B	Clouds and sunshine	2–3 P.M., Oct. 31	50		42	6.14
	C	Clouds and sunshine	2–4 P.M., Oct. 31	50		42	8.55
2		Sunshine	Several hours, Oct. 30	50		42	14.80
3	A	North light	Several weeks	42	0	42	4.81
	B	Sunshine	10–11 A.M., Nov. 1	50		42	6.6
	C	Sunshine	10–12 A.M., Nov. 1	50		42	7.41
	D	Sunshine	12 M.–4 P.M., Nov. 1	50		42	16.56
4		Sunshine	9 A.M.–4 P.M., Nov. 9	50	1	42	14.80
5	A	North light	Several weeks	42	0	42	3.24
	B	Fair	10 A.M.–3 P.M., Dec. 16	50	0.25	50	6.56
	C	Fair	10 A.M.–3 P.M., Dec. 16	50	0.50	50	9.85
	D	Fair	10 A.M.–3 P.M., Dec. 16	50	0.75	50	13.13
	E	Fair	10 A.M.–3 P.M., Dec. 16	50	1.0	50	16.42

while experiment 5 shows the amount of oxygen generated by varying quantities of Chlamydomonas during a five-hour period. It may be readily seen in the last columns of the table that the amount of free oxygen is greatly increased in the sunlight.

TABLE 2

Showing that old culture water free from decomposing materials and containing only a small quantity of free oxygen gradually absorbs additional free oxygen from the surrounding air

EXPERIMENT	LOT	TIME, 1918	CULTURE WATER TESTED	OXYGEN PER LITER	WATER	
			cc.	*cc.*		
1	A	4 P.M., Nov. 9	42	7.97	Tap-water	
	B	4 P.M., Nov. 9	42	7.02	Tank rain-water	
2	A	4 P.M., Nov. 7	42	1.51	Old culture water	Unfiltered
	B	4:15 P.M., Nov. 7	42	2.27	Old culture water	Filtered
	C	5 P.M., Nov. 8	42	4.55	Old culture water	Filtered
	D	4 P.M., Nov. 9	42	6.83	Old culture water	Filtered
	E	11 A.M., Nov. 10	42	7.59	Old culture water	Filtered
	F	5 P.M., Nov. 11	42	7.90	Old culture water	Filtered
3	A	9 A.M., Dec. 17	50	2.62	Old culture water	Unfiltered
	B	10 A.M., Dec. 18	50	4.92	Old culture water	Filtered
	C	10 A.M., Dec. 19	50	6.56	Old culture water	Filtered
	D	9 A.M., Dec. 20	50	6.56	Old culture water	Filtered
4	A	3 P.M., Dec. 23	50	3.10	Old culture water	Filtered
	B	3 P.M., Dec. 24	50	4.96	Old culture water	Filtered
	C	3 P.M., Dec. 25	42	6.64	Old culture water	Filtered
	D	3 P.M., Dec. 26	42	7.75	Old culture water	Filtered
5	A	3 P.M., Dec. 25	42	3.32	Old culture water	Filtered
	B	3 P.M., Dec. 26	42	4.80	Old culture water	Filtered
	C	3 P.M., Dec. 27	42	6.64	Old culture water	Filtered
	D	3 P.M., Dec. 28	42	7.38	Old culture water	Filtered
	E	3 P.M., Dec. 29	42	7.75	Old culture water	Filtered

EXPERIMENTS SHOWING THAT WATER CONTAINING A SMALL AMOUNT OF FREE OXYGEN WILL ABSORB ADDITIONAL FREE OXYGEN FROM THE SURROUNDING AIR IN DARKNESS

It is not only important to determine the amount of oxygen generated by Chlamydomonas in the sunlight, but it is also equally important to determine the amount of oxygen that is absorbed from the air by the culture water when in darkness. When Chlamydomonas and Polytoma were added to the old stable-tea culture water which contained only a small quantity of free oxygen and then the culture was placed in darkness for several days the quantity of oxygen increased in the culture water several cubic centimeters per liter. This is shown in tables 2, 5, and 7. In the absence of light Chlamydomonas does not carry on photosynthesis and consequently does not give off free oxygen.

Clear old stable-tea culture was taken, in some experiments it was filtered and in others it was used unfiltered, and the amount of free oxygen determined at the beginning of each experiment. Then several stender dishes containing about 50 cc. of this water was placed in darkness and at successive intervals of twenty-four hours the contents of a dish was tested for free oxygen. Table 2 shows that the culture water gradullay absorbs free oxygen from the air throughout the three to four days' exposure until it usually amounts to from 7 to 8 cc. to a liter.

EXPERIMENTS SHOWING THAT FEWER MALES IN HYDATINA SENTA ARE PRODUCED IN SUNLIGHT, WHERE PRESUMABLY THE AMOUNT OF FREE OXYGEN IS HIGHER, THAN IN DARKNESS WHERE THE AMOUNT OF FREE OXYGEN IS LOWER

In these experiments large-mouthed bottles about $1\frac{1}{2}$ inches diameter were used. Into each there was put a mixture of 50 to 60 cc. of filtered old stable-tea culture water, Chlamydomonas, and a little Polytoma. In the bottles in the sunlight about 1 to 1.5 cc. Chlamydomonas were put in order that there might be a large quantity of oxygen generated. In the bottles in

darkness not as much Chlamydomonas could be added because much of it would die, probably from lack of oxygen, decompose and befoul the water so as to prevent the normal growth and reproduction of the rotifers. In these experiments ten adult rotifers were put into each bottle of culture water and food and one bottle was placed in a pan of running water in the sunlight and the other bottle was placed in darkness at room temperature. Both of these bottles were left undisturbed for six days, then each was well stirred and a few drops of the liquid immediately taken out and the sex of the rotifers in these drops were recorded.

In table 3 it is seen that in the sunlight the males constituted 8 + per cent of the rotifer population of 1736 individuals, while in the darkness the males constituted 28 + per cent of the population of 1654 individuals.

TABLE 3

Showing that rotifers kept in darkness where there is no production of free oxygen by the Chlamydomonas produce more males than rotifers do which are kept in the sunlight in the midst of considerable quantities of free oxygen that is given off by the Chlamydomonas

EXPERIMENTS	TIME, 1918	1 TO 2 CC. OF CHLAMYDOMONAS AND A FEW DROPS OF POLYTOMA IN 50 CC. CULTURE WATER IN EACH EXPERIMENT					
		Sunlight			Darkness		
		Number of ♀	Number of ♂	Per cent of ♂	Number of ♀	Number of ♂	Per cent of ♂
1	April 9–15	47	8	14+	27	25	48+
2	April 15–21	266	28	9+	284	78	21+
3	April 17–22	171	15	8+	152	67	30+
4	April 17–22	129	26	16+	65	18	21+
5	April 17–23	175	31	15+	223	85	27+
6	April 18–24	37	12	24+	38	12	24+
7	April 20–27	40	2	4+	61	9	12+
8	April 28–May 5	110	9	7+	70	24	25+
9	April 29–May 5	200	4	1+	42	42	50
10	April 30–May 6	150	3	2	83	56	40+
11	May 1– 7	150	2	1+	71	32	31+
12	May 2– 8	65	3	4+	14	8	36+
13	May 10–16	50	3	5+	50	18	26+
Totals		1590	146	8+	1180	474	28+

In a former paper it was shown how a high per cent of males could be obtained by feeding Chlamydomonas in the sunlight, and now opposite results are obtained! These contradictory results are due to differences in manipulations of feeding and also to different conditions of the Chlamydomonas itself as a food in these two instances. In the former experiments the Chlamydomonas were put into the culture water with the rotifers and the rotifers fed upon them for only a few hours, during which the Chlamydomonas were kept actively swimming toward the lighted side of the dish as it was rotated upon a kymograph.

In these later experiments the feeding conditions were quite different. No rotation of the bottles was made, and when this is not done many of the Chlamydomonas swim to the lighted side of the dish and adhere to the surface of the glass. This enables all such individuals to escape from being eaten by the rotifers. Sometimes the rotifers were able to pick up only a small number of stray Chlamydomonas in direct sunlight. On cloudy days and in darkness the Chlamydomonas are more active and are more available as food for the rotifers. In experiment 6 of table 3 cloudy weather prevailed throughout the last five days that the rotifers were in the experiment. Probably the Chlamydomonas remained active in the diffuse daylight so that the rotifers were enabled to feed upon them as readily as in the darkness. This would explain why the per cent of males is equal in each lot.

When the Chlamydomonas remain in the sun for several days in the old stable-tea culture water, which is devoid of all nutritional substances, very little, if any, reproduction occurs among them, but each individual becomes of full size and is covered with a tough covering which renders it indigestible for the rotifers. Consequently, if nearly all of the Chlamydomonas attach themselves to the side of the dish, the rotifers are unable to obtain a superabundance of food or, on the other hand, if some of the Chlamydomonas remain active they develop such a tough covering as to render them nearly unfit as food for the rotifers. In either case the rotifers are not overfed in the sunlight. While, on the contrary, in darkness all of the Chlamydomonas

remain active and small and do not develop the tough covering. Thus there is a fine food supply for the rotifers for several days. The matter of the food supply probably explains the sex ratio. When the supply is good as in darkness more males are produced and when it is poor as in sunlight fewer males are produced. In all of these experiments in the sunlight much free oxygen was given off during the day and rose to the surface forming a frothy scum.

Table 4 shows the light conditions throughout these experiments.

TABLE 4

Showing light conditions of the days during the experiments in table 3

TIME, 1918	LIGHT CONDITIONS DURING THE DAY	TIME, 1918	LIGHT CONDITIONS DURING THE DAY
April 9–14	Sun	April 26–27	Cloudy
April 15	Cloudy	April 28–May 4	Sun
April 16–18	Sun	May 5	Sun and clouds
April 19–24	Cloudy	May 6	Sun
April 25	Sun		

Table 5 shows the details and results of another set of experiments in the sunlight and darkness in which the quantity of free oxygen in the culture water was determined both at the beginning and at the end of many of the experiments. The amount of the food was more accurately measured than in the experiments of table 3. Small amounts of Chlamydomonas, 0.10 cc., and of Polytoma, 0.05 cc., were put into 10 cc. of the culture water and well stirred. Then 3 cc. of this mixture was added to 50 cc. of the filtered old stable-tea culture water. To the lot that was placed in sunlight 0.2 to 1 cc. of additional Chlamydomonas was added. Each lot was divided into three portions and put into three vials, 1 inch diameter, and allowed to stand twenty-four hours and then three or four rotifers were added. These experiments were started in the morning and thus afforded an opportunity for the Chlamydomonas to generate sufficient oxygen in the sunlight to quite thoroughly charge the culture water with it before the rotifers were added. It also enabled many of the Chlamydomonas to become attached to the sides

TABLE 5

Showing that in sunlight where 2 to 15 cc. of free oxygen per liter is present about 43 per cent of male-producing females are produced, while in darkness where only 2 to 8 cc. of free oxygen per liter is present about 78 per cent of male-producing females are produced

EXPERIMENTS	PERIOD	TIME, 1918	DIRECT SUNLIGHT						DARKNESS					
			Culture water tested	Oxygen per liter	Number females treated	Daughters			Culture water tested	Oxygen per liter	Number females treated	Daughters		
						♀♀	♂♀	Per cent of ♂♀				♀♀	♂♀	Per cent of ♂♀
			cc.	cc.	cc.				cc.	cc.				
1	Beginning	10 A.M., Oct. 17			12						12			
	End	10 A.M., Oct. 20				9	11	55				4	16	80
2	Beginning	10 A.M., Oct. 20			3						3			
	End	4 P.M., Oct. 23				29	15	34+				12	25	67+
3	Beginning	10 A.M., Oct. 20			4						4			
	End	11 A.M., Oct. 24				27	13	32+				4	19	82+
4	Beginning	11 A.M., Oct. 21			4						4			
	End	10 A.M., Oct. 25				28	8	22+				15	21	58+
5	Beginning	10 A.M., Oct. 22			5						5			
	End	10 A.M., Oct. 26				28	12	30				7	33	82+
6	Beginning	10 A.M., Oct. 24			4						4			
	End	10 A.M., Oct. 28				9	1	10				1	7	87+
7	Beginning	10 A.M., Oct. 24			4						4			
	End	10 A.M., Oct. 28				10	8	42+				3	17	85
8	Beginning	10 A.M., Oct. 28			16						16			
	End	10 A.M., Oct. 31	42	5.64		17	33	66	42	5.64		15	35	70
9	Beginning	M., Oct. 28			12						12			
	End	10 A.M., Nov. 1	42	5.97		25	25	50	42	4.81		19	31	62
10	Beginning	10 A.M., Oct. 29			12						12			
	End	4 P.M., Nov. 2	42	12.11		35	15	30	42	5.33		14	36	72
11	Beginning	10 A.M., Oct. 30			12						12			
	End	5 P.M., Nov. 3	42	11.72		10	38	79+	42	5.86		9	34	79+
12	Beginning	10 A.M., Oct. 31			12						12			
	End	5 P.M., Nov. 4	42	7.81		24	26	52	42	6.25		4	46	92

TABLE 5—*Concluded*

EXPERIMENTS	PERIOD	TIME, 1918	DIRECT SUNLIGHT						DARKNESS					
			Culture water tested	Oxygen per liter	Number females treated	Daughters			Culture water tested	Oxygen per liter	Number females treated	Daughters		
						♀♀	♂♀	Per cent of ♂♀				♀♀	♂♀	Per cent of ♂♀
			cc.	*cc.*					*cc.*	*cc.*				
13	Beginning	9 A.M., Nov. 1	42	2.15	12				42	2.15	12			
	End	3 P.M., Nov. 5	42	5.86		30	20	40	42	5.33		2	48	96
14	Beginning	9 A.M., Nov. 2	42	0.97	12				42	0.97	12			
	End	3 P.M., Nov. 6	42	7.81		33	17	34	42	5.93		6	44	88
15	Beginning	10 A.M., Nov. 3	42	2.65	12				42	2.65	12			
	End	3 P.M., Nov. 7	42	7.44		14	36	72	42	6.45		3	47	94
16	Beginning	8 A.M., Nov. 10	42	3.03	13				42	3.03	13			
	End	M., Nov. 14	42	11.72		28	22	44	42	7.42		12	38	76
17	Beginning	8 A.M., Nov. 11	42	4.31	13				42	4.31	13	1		
	End	3 P.M., Nov. 15	42	11.72		32	18	36	42	7.81		10	40	80
18	Beginning	11 A.M., Nov. 12	42	3.59	13				42	3.59	13			
	End	4 P.M., Nov. 16	42	7.81		32	18	36	42	7.81		9	41	82
19	Beginning	10 A.M., Nov. 13	42	3.50	13				42	3.50	13			
	End	M., Nov. 17	42	8.21		27	23	46	42	8.21		11	39	78
20	Beginning	9 A.M., Nov. 14	42	3.12	13				42	3.12	13			
	End	4 P.M., Nov. 18	42	15.63		36	14	28	42	7.81		18	32	64
Summary				9+*		483	373	43+		6+*		178	649	78+

* Average at end.

of the vials and thus render the available food supply scarce for the rotifers when they were added at the end of twenty-four hours. Three days after the rotifers were added about fifty young females were isolated in watch-glasses and the sex of their offspring recorded.

In many of the experiments the Chlamydomonas in the sunlight had so spent themselves at the end of four days that no more free oxygen was found in the culture water than was found

in the culture water of the lots in the darkness. In others, however, a high per cent of oxygen was found at the end of four days. Several determinations of free oxygen were made in the mornings of cultures similar to the above and a small quantity of free oxygen was always found as in experiments 8 and 9. Thus showing that the excess quantity of free oxygen escapes from the culture water during the night and in the mornings no more is found than would have been found in such culture waters if exposed merely to the air. In the cultures in the sunlight much free oxygen was generated during the first three days, which was very evident by the frothy scum on the surface of the water. During this time the rotifers were subjected for many hours each day to a high per cent of free oxygen. During the night the excess of oxygen gradually escaped, but in the sunlight of the following day a new excess of oxygen was generated.

The rotifers in the sunlight were subjected to perhaps 10 to 15 cc. of oxygen per liter for a period each day, while the rotifers in the darkness were never subjected to more than was absorbed by the water from the air, 7 to 8 cc. of oxygen per liter. Bearing this in mind, it is of considerable interest to compare the sunlight lots with the darkness lots in individual experiments as in 10, 17, and 20 of table 5 or to compare the average results of the total summary of all the experiments. In all the experiments excepting no. 11 the per cent of male-producing females is much lower in sunlight where there is an excess of free oxygen than it is in darkness where there is no more free oxygen than can be absorbed from the air. The results of experiment 11 are not clear, inasmuch as there was brilliant sunlight throughout the four days of the experiment.

Table 6 shows the light conditions throughout these experiments of table 5.

TABLE 6

Showing light conditions of the days during the experiments of table 5

TIME, 1918	LIGHT CONDITIONS DURING THE DAY	TIME, 1918	LIGHT CONDITIONS DURING THE DAY
October 17	Sun	Oct. 29–Nov. 3	Sun
October 18–19	Cloudy	November 4	Clouds and sun
October 20–21	Sun	November 5–7	Cloudy
October 22	Clouds and sun	November 8–9	Sun
October 23	Cloudy	November 10	Clouds and sun
October 24	Sun	November 11–13	Sun
October 25–27	Cloudy	November 14	Clouds and sun
October 28	A.M., sun P.M., cloudy	November 15	Cloudy
		November 16–19	Sun

EXPERIMENTS SHOWING THAT CULTURE WATER CONTAINING A VERY LOW PERCENTAGE OF FREE OXYGEN YIELDS AS MANY MALE-PRODUCING FEMALES AS CULTURE WATER CONTAINING A MUCH HIGHER PERCENTAGE OF FREE OXYGEN

The experiments of the preceding tables 1 to 6 may be of interest, but the crucial test of the effect of oxygen in causing male-producing females to appear is really made in these experiments in table 7. All of these experiments were carried on in darkness and the food and culture-water conditions were the same as those in the darkness experiments of table 5 with the exception that the culture water containing the food was put into one $1\frac{1}{4}$-inch vial instead of being divided and put into three vials. The vials in lots A were not stoppered, but were kept open so that the surrounding air came into contact with the surface of the water, but the vials of lots B were closed with tightly fitting ground-glass stoppers. A small quantity of air, 3 cc. to 0.5 cc., was left in each vial. In some experiments the vials in lots B were inverted. The inclosed air bubble was changed every morning and evening, otherwise both the food and the rotifers would soon have died from lack of a sufficient oxygen supply.

Thirteen female rotifers were put into each vial at the beginning of each experiment and allowed to remain three days undisturbed. At the end of that time, fifty young females were

selected at random and isolated in watch-glasses. In some experiments there were fewer than fifty young daughter females produced, and in such cases all of the young females were isolated as in lots B of experiments 13 and 14. An equal number of young females were isolated from the control lots A.

The quantity of free oxygen in the culture water was determined both at the beginning and at the end of each experiment in both lots A and lots B. Old culture water was used which was made about the middle of the previous August and which contained only a small quantity of free oxygen. After this culture water was filtered it absorbed additional free oxygen from the air in lots A, while in lots B, in the stoppered vials, the quantity of free oxygen was diminished when the air bubble was made small enough as in lots B of experiments 9 to 17 and 19. In experiments 9, 10, 12, 13, 14, and 18 the oxygen supply became so low in lots B that none of the rotifer eggs hatched until the vials were opened and additional oxygen was supplied. In lots B of experiments 8, 11, 15, 16, 17, and 19 fewer eggs had hatched than in lots A, in all of which there were more than fifty young females at the end of three days.

In lots A the free oxygen increased by absorption from the air to from 6 to 8 cc. per liter, while in lots B it ranged from 6 to 1 cc. per liter at the end of the experiments. However much the two lots, A and B, varied in their oxygen content, the per cent of male-producing females produced was about equivalent in each lot of the individual experiments and also in the general average of the summary of all of the experiments. In fact, the per cent of male-producing females in the summary of all lots A and lots B is practically identical. Such equivalent results in two parallel lots of rotifers, even under the same conditions, never previously have been obtained by the author.

The most striking result of these experiments is the production of such a high per cent of male-producing females in culture water that was nearly depleted of free oxygen. The highest per cent among fifty young females was ninety-two. It is recorded in lot B of experiment 5. Only two or three lots exposed to the air exceeded this.

TABLE 7

Showing the rotifers in culture water containing the minimum of 1 to 3 cc. of free oxygen per liter produced as many male-producing females, 74 per cent, as rotifers produced in culture water containing 2 to 7 cc. of free oxygen per liter

				CONTROL IN DARKNESS						DIMINISHED AIR SUPPLY								
	LOTS	PERIOD.	TIME, 1918	Air supply	Culture water tested	Oxygen per liter	Number of females treated	Daughters ♀ ♀	Daughters ♂ ♀	Daughters Per cent of ♂ ♀	Lots	Air supply	Culture water tested	Oxygen per liter	Number of females treated	Number eggs hatched at end of 3 days' treatment	Daughters ♀ ♀	Daughters ♂ ♀	Daughters Per cent of ♂ ♀
					cc.	cc.						cc.	cc.	cc.					
1	A	Beginning	4 P.M., Nov. 14	Open dish	42	5.08	13				B	3.0	42	5.08	13				
		End	10 A.M., Nov. 17	Open dish	42	7.81		9	41	82	B	3.0	42	5.47		50+	11	39	78
2	A	Beginning	4 P.M., Nov. 15	Open dish	42	3.52	13				B	3.0	42	3.52	13				
		End	3 P.M., Nov. 18	Open dish	42	7.81		6	44	88	B	3.0	42	5.86		50+	17	33	66
3	A	Beginning	9 A.M., Nov. 16	Open dish	42	3.52	13				B	3.0	42	3.52	13				
		End	4 P.M., Nov. 19	Open dish	42	7.03		2	48	96	B	3.0	42	5.08		50+	8	42	84
4	A	Beginning	10 A.M., Nov. 17	Open dish	42	4.30	13				B	1.5	42	4.30	13				
		End	5 P.M., Nov. 20	Open dish	42	7.81		13	37	74	B	1.5	42	6.64		50+	9	41	82
5	A	Beginning	3 P.M., Nov. 18	Open dish	42	2.93	13				B	1.5	42	2.93	13				
		End	3 P.M., Nov. 21	Open dish	42	7.42		3	47	94	B	1.5	42	3.50		50+	4	46	92
6	A	Beginning	3 P.M., Nov. 19	Open dish	42	3.50	13				B	1.0	42	3.50	13				
		End	5 P.M., Nov. 22	Open dish	42	7 81		20	30	60	B	1.0	42	3.50		50+	8	42	84

7	A	Beginning	4 P.M., Nov. 20	Open dish	42	2.73	13				B	1.0	42	2.73	13				
		End	5 P.M., Nov. 23	Open dish	42	7.38		15	35	70	B	1.0	42	3.32		50+	14	36	72
8	A	Beginning	3 P.M., Nov. 21	Open dish	42	3.50	13				B	0.5	42	3.50	13				
		End	M., Nov. 24	Open dish	42	7.38		22	28	56	B	0.5	42	4.43		44	13	31	70+
9	A	Beginning	6 P.M., Nov. 22	Open dish	42	3.50	13				B	0.5	42	3.50	13				
		End	4 P.M., Nov. 25	Open dish	42	7.38		10	40	80	B	0.5	42	3.32		0	5	25	83+
10	A	Beginning	10 A.M., Nov. 23	Open dish	42	3.24	13				B	0.5	42	3.24	13				
		End	4 P.M., Nov. 26	Open dish	42	7.38		8	42	84	B	0.5	42	2.21		0	12	38	76
11	A	Beginning	1 P.M., Nov. 24	Open dish	42	4.80	13				B	0.5	42	4.80	13				
		End	3 P.M., Nov. 27	Open dish	42	7.38		23	27	54	B	0.5	42	2.21		20	26	44	62+
12	A	Beginning	9 A.M., Nov. 26	Open dish	42	2.58	13				B	0.5	42	2.58	13				
		End	3 P.M., Nov. 29	Open dish	42	7.83		1	15	93+	B	0.5	42	1.47		0	10	5	33+
13	A	Beginning	11 A.M., Nov. 28	Open dish	42	2.58	13				B	0.5	42	2.58	13				
		End	10 A.M., Dec. 1	Open dish	42	8.85		7	43	86	B	0.5	42	1.02		0	3	22	88
14	A	Beginning	3 P.M., Nov. 29	Open dish	42	3.06	13				B	0.5	42	3.06	13				
		End	3 P.M., Dec. 2	Open dish	42	6.47		12	13	52	B	0.5	42	2.04		0	13	12	48
15	A	Beginning	9 A.M., Nov. 30	Open dish	42	3.40	13				B	0.5–1	42	3.40	13				
		End	3 P.M., Dec. 3	Open dish	42	6.81		30	20	40	B	0.5–1	42	1.70		15	14	31	68+
16	A	Beginning	11 A.M., Dec. 1	Open dish	42	4.08	13				B	0.5	42	4.08	13				
		End	4 P.M., Dec. 4	Open dish	42	6.13		7	43	86	B	0.5	42	2.67		6	15	19	55+

TABLE 7—*Concluded*

	LOTS	PERIOD	TIME, 1918	CONTROL IN DARKNESS						DIMINISHED AIR SUPPLY							
				Air supply	Culture water tested	Oxygen per liter	Number of females treated	Daughters ♀ ♀	Daughters ♂ ♀	Daughters Per cent of ♂ ♀	Lots	Air supply	Culture water tested	Oxygen per liter	Number of females treated	Number eggs hatched at end of 3 days treatment	Daughters ♀ ♀	Daughters ♂ ♀	Daughters Per cent of ♂ ♀
					cc.	*cc.*						*cc.*	*cc.*	*cc.*					
17	A	Beginning	9 A.M., Dec. 2	Open dish	42	2.67	13				B	0.5	42	2.67	13				
		End	4 P.M., Dec. 5	Open dish	42	7.49		10	40	80	B	0.5	42	1.70		12	9	41	82
18	A	Beginning	11 A.M., Dec. 3	Open dish	42	2.67	13				B	0.5–1	42	2.67	13				
		End	1 P.M., Dec. 6	Open dish	42	7.15		11	29	72+	B	0.5–1	42	3.06		0	7	35	83+
19	A	Beginning	11 A.M., Dec. 4	Open dish	42	2.38	13				B	0.5–1	42	2.38	13				
		End	4 P.M., Dec. 7	Open dish	42	7.15		12	38	76	B	0.5–1	42	2.67		28	14	36	72
Total								221	660	74+							212	618	74+
Summary		Average				5.38								3.31					
		Average at beginning				3.37								3.37					
		Average at end				7.39								3.25					

EXPERIMENTS SHOWING THAT THE FREE OXYGEN CONTENT OF WATER IS NOT INCREASED WHEN CHLAMYDOMONAS IS ADDED

After the preceding experiments had been completed, it was realized that perhaps there was a sufficient amount of free oxygen retained within the individual cells themselves of Chlamydomonas to exert an appreciable influence when the Chlamydomonas were transferred to other culture waters. In order to test this possibility, the experiments recorded in table 8 were performed.

The Chlamydomonas was allowed to remain in the sunlight for several hours; then it was centrifuged, its culture water drained off, sufficient quantity of water added to liquefy it, and definite quantities of it added to various kinds of water. The water used was mainly rain-water, which varied widely in the free oxygen content. The rain-water that had been standing in the pipes from a large storage tank contained less than 1 cc. of free oxygen per liter, while rain-water in battery jars which had been exposed to the air for several days contained as much as 7 or 8 cc. of free oxygen per liter. Chlamydomonas was added to these two kinds of rain-water. Oxygen tests were made immediately with some unfiltered lots containing Chlamydomonas, while other lots were first filtered before being tested. In a few experiments Chlamydomonas was allowed to remain in the water about ten minutes, while in others it was allowed to remain about four hours before the tests were made.

It was found that a considerable error was introduced by filtration. The quantity of oxygen was increased even in the most hurried filtration and was increased very markedly if the filtering process was prolonged for a few minutes, especially in the water that contained a very small quantity of free oxygen at the beginning of the experiment. In some experiments the water was decanted before testing for oxygen, but this method was only feasible when a sufficient time had elapsed to allow the Chlamydomonas to settle to the bottom of the bottle.

In none of these experiments, when the error due to filtration was taken into consideration, was there found any evidence to

TABLE 8

Showing that when masses of Chlamydomonas which have been standing in the sun for several hours are put into rain water or old culture water the oxygen content of these waters is not increased

EXPERIMENTS	LOTS	TIME, 1919	WATER	WATER CONDITIONS	WATER USED	CHLAMYDOMONAS ADDED	OXYGEN PER LITER	LIGHT CONDITIONS OF CHLAMYDOMONAS
					cc.	cc.	cc.	
1	A	4 P.M., Jan. 4	Rain-water standing in pipes	Unfiltered	42	0	0.73	
	B	4 P.M., Jan. 4	Rain-water standing in pipes	Unfiltered	42	0.5	0.73	Sun during day
2	A	8:30 A.M., Jan. 6	Rain-water standing in jar 36 hours	Unfiltered	42	0	6.64	
	B	8:30 A.M., Jan. 6	Rain-water standing in jar 36 hours	Unfiltered	42	1.0	5.16	Darkness of preceding night
3	A	4 P.M., Jan. 6	Rain-water standing in jar several days	Unfiltered	42	0.0	7.38	
	B	4 P.M., Jan. 6	Rain-water standing in jar several days	Unfiltered	42	1.0	5.16	Sun during day
4	A	4 P.M., Jan. 7	Rain-water standing in jar 72 hours	Unfiltered	42	0.0	7.38	
	B	4 P.M., Jan. 7	Rain-water standing in jar 72 hours	Unfiltered	42	0.5	5.53	Sun during day
	C	4 P.M., Jan. 7	Rain-water standing in jar 72 hours	Filtered 10 minutes after addition of Chlamydomonas	42	0.5	5.74	Sun during day

5	A	5 P.M., Jan. 7	Rain-water standing in pipes	Unfiltered	42	0.0	0.36	
	B	5 P.M., Jan. 7	Rain-water standing in pipes	Unfiltered	42	0.0	0.36	
	C	5 P.M., Jan. 7	Rain-water standing in pipes	Filtered	42	0.0	1.44	
	D	5 P.M., Jan. 7	Rain-water stanidng in pipes	Filtered	42	0.0	1.10	
6	A	6 P.M., Jan. 7	Rain-water standing in jar 72 hours	Unfiltered	42	0.0	7.19	
	B	6 P.M., Jan. 7	Rain-water in standing jar 72 hours	Unfiltered	42	0.0	7.16	
	C	6 P.M., Jan. 7	Rain-water standing in jar 72 hours	Filtered	42	0.0	7.53	
	D	6 P.M., Jan. 7	Rain-water standing in jar 72 hours	Filtered	42	0.0	7.38	
7	A	4 P.M., Jan. 8	Rain-water standing in jar 96 hours	Unfiltered	42	0.0	7.38	
	B	4 P.M., Jan. 8	Rain-water standing in jar 96 hours	Unfiltered	42	0.5	5.74	Sun during day
	C	4 P.M., Jan. 8	Rain-water standing in jar 96 hours	Filtered 5 minutes after addition of Chlamydomonas	42	0.5	6.64	Sun during day
8	A	3 P.M., Jan. 9	Rain-water standing in pipes	Unfiltered	42	0.0	0.22	
	B	3 P.M., Jan. 9	Rain-water standing in pipes	Filtered	42	0.0	1.84	
	C	3 P.M., Jan. 9	Rain-water standing in pipes	Unfiltered	42	0.5	0.14	Sun during day
	D	3 P.M., Jan. 9	Rain-water standing in pipes	Filtered at once after addition of Chlamydomonas	42	0.5	1.84	Sun during day

TABLE 8—*Continued*

EXPERIMENTS	LOTS	TIME, 1919	WATER	WATER CONDITIONS	WATER USED	CHLAMYDOMONAS ADDED	OXYGEN PER LITER	LIGHT CONDITIONS OF CHLAMYDOMONAS
					cc.	*cc.*	*cc.*	
9	A	3 P.M., Jan. 10	Old culture water	Decanted	42	0.0	0.96	
	B	3 P.M., Jan. 10	Old culture water	Filtered	42	0.0	1.62	
	C	3 P.M., Jan. 10	Old culture water	Decanted	42	0.5	0.36	Sun during day
	D	3 P.M., Jan. 10	Old culture water	Filtered 10 minutes after addition of Chlamydomonas	42	0.5	1.69	Sun during day
10	A	4 P.M., Jan. 11	Rain-water standing in pipes	Unfiltered	42	0.0	0.57	
	B	4 P.M., Jan. 11	Rain-water standing in pipes	Filtered	42	0.0	2.36	
	C	4 P.M., Jan. 11	Rain-water standing in pipes	Decanted 4 hours after addition of Chlamydomonas	42	0.5	0.36	Sun during A.M.
	D	4 P.M., Jan. 11	Rain-water standing in pipes	Filtered 4 hours after addition of Chlamydomonas	42	0.5	2.21	Sun during A.M.
11	A	4 P.M., Jan. 11	Rain-water standing in pipes	Unfiltered	42	0.0	7.60	
	B	4 P.M., Jan. 11	Rain-water standing in pipes	Decanted 4 hours after addition of Chlamydomonas	42	0.5	5.16	Sun during A.M.
	C	4 P.M., Jan. 11	Rain-water standing in pipes	Filtered 4 hours after addition of Chlamydomonas	42	0.5	5.53	Sun during A.M.

support the contention that appreciable quantities of free oxygen may be introduced into the new water with or within the cells of Chlamydomonas.

In the experiments in darkness of table 7 only 0.05 or 0.10 cc. of Chlamydomonas was used which, of course, would have shown lesser results in regard to oxygen if they had been tested than the present experiments in which 0.5 cc. of Chlamydomonas was used.

DISCUSSION

In the recent work by Shull the summary of the results of the experiments under normal air conditions and under the 40 per cent and 60 per cent oxygen conditions show a higher per cent of male-producing females produced under the 40 per cent and 60 per cent oxygen conditions than under air conditions. If, however, one examines closely the individual experiments or lots in the tables 1, 2, and 5 of the results it is readily seen that the higher per cent under the oxygen conditions is produced in table 1 by the extraordinary results of two out of the six experiments. In table 2 three experiments out of fourteen experiments causes the higher per cent of male-producing females to be obtained. In table 5 two experiments out of twenty experiments of the oxygen-treated ones caused the total average per cent of male-producing females to be twice as large as it would have been without these two experiments.

Some of the exceptionally favorable experiments under oxygen conditions were paralleled with similar results under air conditions in the controls. Thus indicating that the high per cent of male-producing females produced in parallel lots in air and in oxygen may have been due to other influences than an excess of oxygen. In some of the experiments under oxygen conditions no male-producing females at all were produced, while in many others very few were produced. If the oxygen was a real influential factor in causing male-producing females to be produced, many ought to have been produced in every experiment.

Shull, however, does not claim that oxygen is the only factor that causes an increase in male-producing females, but that,

nevertheless, it is one of the potent factors in causing male-producing females to increase in number. The author takes the opposite view-point that oxygen is not influential in causing an increase of the male-producing females.

In the experiments of Shull under air conditions, the rotifers produced 0 to 52 per cent of male-producing females in individual experiments and the average in the grand total production of 2334 females in tables 1, 2, and 5 to 8 was 10 + per cent of male-producing females. This per cent was of those females produced during the first twenty-four hours of the experiments. According to sample tests of such culture water as constituted these experiments, the quantity of free oxygen present during the twenty-four hour period was 5 + to 4 + cc. per liter. These results comprised of the production of about 10 per cent male-producing females in culture water containing 5 + to 4 + cc. of free oxygen per liter should now be compared with the author's experiments, lots B in table 7. In these lots with the diminished air supply the quantity of free oxygen at the end of the three-day period of the individual experiments was in some instances 1 + cc. per liter. The average quantity of free oxygen in all of the lots at the end was 3 + cc. per liter. This was a lesser quantity than was found in the experiments of Shull in the air. Shull obtained an average of 10 + per cent male-producing females in culture water containing 5 to 4 + cc. of oxygen per liter, while the author obtained an average of 74 + per cent of male-producing females in culture water containing 3 + cc. of oxygen per liter. In individual lots B of experiments 13, 17, and 19 in which the free oxygen was never more than 2 cc. per liter throughout the experiment and in lots B of experiments 13 and 17 in which the oxygen was diminished from 2 + cc. to 1 + cc. per liter during the experiment, the per cent of male-producing females ranged from 72 to 88. If these lots B of experiments 13, 17, and 19 are compared with the parallel lots A of the same experiments in which the quantity of free oxygen ranges from 3 + cc. to 8 + cc. per liter during the three-day period of the experiments, it is seen that in this increased quantity of free oxygen there is no increase in the percentage of

male-producing females. Furthermore, if the total averages are compared, it is seen that the high percentages of male-producing females are identical, although the quantity of free oxygen at the end of the three-day period averages in lots A at 5+ cc. per liter and in lots B at 3 + cc. per liter. Thus demonstrating that the production of male-producing females does not depend directly upon the presence of appreciable quantities of free oxygen in the culture water.

SUMMARY

1. In the sunlight free oxygen in considerable quantities is given off by the green flagellates, Chlamydomonas.

2. In darkness no free oxygen is given off by the Chlamydomonas.

3. No appreciable quantity of free oxygen was found to be contained within the individual cells of Chlamydomonas when they were transferred from their original culture water into other water.

4. Culture water free from decomposing materials absorbs free oxygen from the surrounding air until its capacity of from 7 to 8 cc. per liter is attained.

5. In the sunlight fewer male rotifers and also fewer male-producing female rotifers are produced in culture water containing Chlamydomonas which have given off much free oxygen than are produced in darkness in culture water containing less free oxygen. This is due to the fact that in the sunlight the Chlamydomonas become less available as food for the rotifers, while in darkness they remain more available for food throughout several days and nights.

6. Culture water containing the minimum quantity (in some cases less than the minimum quantity) of free oxygen, 1 cc. to 3 cc. per liter, in order to allow the normal activities of the rotifers, yields as many male-producing females as culture water containing from 2 to 8 cc. of oxygen per liter.

7. The general conclusion is that oxygen is a factor in causing a production of males except inasmuch as it is necessary for all life processes and activities of the rotifers.

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